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Sex-associated expression of mouse hepatic and renal CYP2B enzymes by glucocorticoid hormones

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Abstract

The expression of *Cyp2b9* and *Cyp2b10* genes was investigated in kidney, liver, and cultured hepatocytes of adult C57BL/6NCrj mice. The constitutive expression level of CYP2B mRNA in kidney was higher in female than in male mice, as it was in the liver where more CYP2B9 than CYP2B10 was expressed in the females, and more CYP2B10 was expressed in the males. After treatment with dexamethasone (Dex), induction of CYP2B10 mRNA and protein in the kidneys was far greater in male than in female mice. In contrast to Dex, phenobarbital (PB), pregnenolone-16α-carbonitrile (PCN), and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) did not induce the expression of the *Cyp2b* gene in the kidneys of either sex. In the liver, PB, PCN, and DDT induced both CYP2B9 and CYP2B10 in both sexes to the same extent, whereas Dex induced only CYP2B10 and simultaneously suppressed CYP2B9. Dex-inducible expression of CYP2B mRNA was decreased by 11β-[4-dimethylamino]phenyl-17β-hydroxy-17-[1-propynyl]estra-4,9-dien-3-one (RU-486), in both the kidneys and liver from male mice, and in cultured hepatocytes. However, RU-486 itself induced the expression of CYP2B mRNA in female liver and cultured hepatocytes. Interestingly, RU-486 increased PB-inducible expression of these species in cultured hepatocytes. Gonadectomy increased the expression of CYP2B mRNA in untreated male liver, but suppressed Dex-induced expression in the kidneys of both sexes. These observations suggest that (a) there are multiple regulatory pathways in the expression of *Cyp2b* genes, one of which used by Dex is mediated via the glucocorticoid receptor, which is different from that used by PB, and (b) sex hormones play a role in the regulation of the sex-dependent expression of *Cyp2b* genes in the mouse.

Keywords: Glucocorticoid; Phenobarbital; RU-486; Pregnenolone-16α-carbonitrile; DDT; *Cyp2b9/10* genes

1. Introduction

The P450 genes encode a superfamily of heme-thiolate proteins responsible for the oxidative metabolism of chemically diverse compounds of both endogenous and exogenous origin [1,2]. In some cases, the toxicity of certain xenobiotics, such as mutagens and carcinogens, is enhanced by P450-dependent metabolism. Expression of P450 is influenced by endocrine factors [3,4], such as growth hormone, sex hormones, and glucocorticoid hormones. CYP2B1 and CYP2B2 in rats, as well as CYP2B9 and CYP2B10 in mice, are major CYP2B isoenzymes expressed constitutively and inducibly [5–20]. Their hepatic constitutive expression is sexually dimorphic, namely male > female in rats and female > male in mice [17–20]. This sexual dimorphism in rats for CYP2B1 and CYP2B2 can be explained by a sex-dependent secretion of growth hormone, i.e. expression is suppressed more in female than in male rats by growth hormone [16,21–24]. However, a previous study did not support the notion that the secretion profile of growth hormone universally explains the sex-dependent difference in mRNA expression levels [17], although hypophysectomy has been shown to increase the expression of CYP2B mRNA in male mouse liver [16]. Therefore, al-

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Abbreviations: Dex, dexamethasone; PB, phenobarbital; PCN, pregnenolone-16α-carbonitrile; DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane; P450, cytochrome P450; CAR, constitutive androstane receptor; PBREM, phenobarbital-responsive enhancer module; GR, glucocorticoid receptor; GRE, glucocorticoid-responsive element; PXR, pregnane X receptor; RT–PCR, reverse transcriptase–polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NR1, nuclear receptor binding site 1; SSC, standard saline citrate; and RXR, retinoid X receptor.

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though the secretion profile of growth hormone in mice is the same as that in rats, this explanation is not applicable to the dimorphism observed in the case of *Cyp2b9* and *Cyp2b10* genes in mice.

PB, PCN, and DDT, as well as Dex, a synthetic glucocorticoid, have been shown to modulate the expression of the CYP2B subfamily in murine liver [16–20,24–26]. In rats, PB induced hepatic expression of some CYP2B subfamily isoform mRNAs [8,10,11,16,19], whereas it did not evoke renal expression of these isoforms [27–30]. Additionally, no comparative information about the different actions of PB on these two organs in mice was obtained. However, by using a mouse-model system, the regulatory mechanism involving PB has been identified to occur through a key regulatory factor, the liver-enriched orphan nuclear receptor CAR. CAR interacts with the PBREM, a PB-responsive element that is localized in the 5′-flanking region of the *Cyp2b10* gene, and confers PB-inducible *Cyp2b10* gene transcription [31–34]. However, although PBREM seems to be a general PB-responsive enhancer because of its responsiveness to numerous PB-type inducers, a presumable PB receptor or a cellular molecule where PB directly interacts has yet to be identified [34]. Dex also affects *CYP2B* gene expression [20], and the presence of a functional glucocorticoid response element in the *CYP2B2* gene that might play a role in the well-established Dex dependency of PB induction has been reported [35,36]. However, the regulatory mechanism used by Dex appears to be complicated [19,31, 35,37]. One possibility is that Dex induces these isoforms by a mechanism different from that involving the classical GR pathway [19,35,37]. In this regard, several GREs were predicted recently in the 5′-flanking region of the rat *CYP2B2* gene [35] and the mouse *Cyp2b10* gene [32]. However, these findings do not explain the regulatory mechanism of Dex completely. Receptor molecules involved in the Dex regulation of liver CYP2B isoforms have not been clearly established, although other Dex-inducible P450 genes, such as *CYP3A1*, *CYP3A2*, and *CYP3A23*, were suggested to be mediated via the orphan nuclear receptor PXR [25,38]. Since Dex is widely used as a clinical drug and administered concurrently with medications, information related to the Dex-inducible CYP2B subfamily is valuable. For example, it is important to delineate how sex-specific expression of *Cyp2b9* and *Cyp2b10* genes is regulated or modified by endocrine factors.

This study deals with the constitutive and inducible expression of *Cyp2b9* and *Cyp2b10* genes in mouse kidney and liver, as well as in cultured hepatocytes. Dex induced only CYP2B10 in the kidneys of both sexes, although the level of induction was higher in males than females. In contrast to Dex, PB and compounds know to mimic PB (e.g. DDT and PCN) did not induce the expression of the *CYP2B* gene in kidneys, but did induce both *CYP2B9* and *CYP2B10* genes in liver independent of gender. These observations suggest that the mechanism by which mouse CYP2B expression is regulated by Dex differs somewhat from that of the other inducers.

2. Materials and methods

2.1. Chemicals

Materials for culturing hepatocytes were purchased from ICN Biomedicals Inc., Collaborative Research Inc., and Kyokuto Seiyaku. Percoll and collagenase (Type I) were products of Pharmacia Biotech AB and the Sigma Chemical Co., respectively. A partial cDNA clone of mouse CYP2B10 was a gift from Dr. M. Negishi, NIEHS. Restriction endonucleases, an RNA PCR kit version 2.1, and T4 polynucleotide kinase were obtained from TaKaRa Biomedicals. [α-^{32}P]dCTP (3000 Ci/mol) was a product of ICN Biomedicals. The Amersham Pharmacia Biotech Co. supplied the [γ-^{32}P]ATP (6000 Ci/mol) and membranes for blotting (Hybond-C and Hybond-N). Oligonucleotides for PCR primers and the gel-shift assay were provided by Hokkaido System Sciences. All other laboratory chemicals were of the highest purity and from commercial suppliers.

2.2. Animals

C57BL/6NCrj mice (Charles River) weighing 20–25 g, three mice per experimental group, were once daily given, subcutaneously, Dex (3, 10, 30, and 100 mg/kg/day), 11β-[4-dimethylamino]phenyl-17β-hydroxy-17-[1-propynyl] estra-4,9-dien-3-one (RU-486) (10 mg/kg/day), PCN (100 mg/kg/day) [39], and DDT (100 mg/kg/day) [24] in corn oil three times. PB was administered intraperitoneally in PBS three times at a dose of 100 mg/kg/day. The control group was simply left untreated because the vehicles did not change CYP2B expression significantly. Gonadectomy and sham operation were performed in 6-week-old mice of both sexes, and 2 weeks after the operation the animals were injected subcutaneously with Dex (10 mg/kg/day) three times. The mice were decapitated 24 hr after the last injection. Total RNA and microsomes were prepared from the kidneys and livers as described [17,20,40].

2.3. Preparation of primary hepatocyte cultures

The liver of each C57BL/6NCrj mouse was perfused with collagenase, and viable hepatocytes were isolated by means of Percoll isodensity centrifugation as described [20, 41,42]. Standard culture conditions were used as follows: the cells were dispersed in Waymouth MB 752/1 medium containing bovine serum albumin (2 g/L), insulin (0.5 mg/L), transferrin (0.5 mg/L), and selenium (0.5 μg/L), and seeded in dishes at a density of 5×10^6 cells/10 mL/100-mm uncoated dish. The Waymouth medium did not contain phenol red, a pH indicator, to exclude estrogen-like action. Because of a lack of cell attachment factors, the

hepatocytes did not spread, although they anchored to the dishes within 24 hr and subsequently aggregated. The aggregates gradually grew and detached to form floating spherical aggregates (spheroids) after day 3 of cultivation. The culture dishes were maintained at 37° in a CO_2-humidified incubator. The medium was renewed daily for 2 days. However, since medium change by itself provokes the expression of some P450 species [43,44], incubation with either inducers or inhibitors was started 24 hr after the last medium change. Then the cells were harvested after another 24 hr to prepare RNA. These culture conditions were convenient for maintaining CYP2B9 and CYP2B10 expression [17,20,40].

2.4. Hybridization of mRNA with P450 probes

Northern blotting was performed as described previously [20,42,43]. Hybridization was carried out at 42° overnight in a mixture containing 50% formamide, 1× Denhardt's solution, 5× SSC, 50 mM sodium phosphate, pH 6.4, salmon testis DNA at 0.25 mg/mL, and a ^{32}P-labeled cDNA probe. Membranes were washed twice for 15 min with 2× SSC and 0.2% SDS, as well as 0.2× SSC and 0.2% SDS at 65°. The blots were exposed to Fuji x-ray films at −80° with an intensifying screen (DuPont). The northern blot data were quantified by a BAS2000 Image Analyser after the membrane-exposed imaging plate was scanned by a BAS2000 Scanner. Since CYP2B9 and CYP2B10 mRNAs are very similar in both nucleotide sequence and size [17, 18], they cannot be detected separately. Therefore, the mRNA detected on the northern blots is referred to as CYP2B.

2.5. RT–PCR

Total RNA was reversed-transcribed using random hexamer primers, and cDNAs for mouse CYP2B9 and CYP2B10 were amplified under the incubation conditions recommended by the supplier (TaKaRa Biomedicals) of the RNA PCR kit version 2.1. Specific oligonucleotide primers were selected according to Nemoto and Sakurai [17] using "Primer Detectives" software (Clontech Laboratories). The sense primer for both CYP2B9 and CYP2B10 was 5′-CTCTTCCAGTGCATCAC-3′ and the antisense primer was 5′-CAATGTAGTCGAGGAGTTCC-3′. The latter was end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. The product length was 229 bases, spanning the region from nucleotide 511 to nucleotide 739 of the open reading frame. To verify which mRNA species was expressed, the PCR products were digested with a restriction enzyme and then resolved by 10% PAGE. CYP2B9 and CYP2B10 were digestible with *Bgl*II and *Hha*I, respectively. The gel was exposed to an x-ray film. To normalize the RNA quantity applied, portions of the cDNA samples were amplified simultaneously by PCR using primers for GAPDH cDNA. The nucleotide sequence of the sense primer was 5′-TCCACTCACGGCAAATTCAACG-3′ and that of the antisense primer was 5′-TAGACTCCACGACATACTCAGC-3′. The PCR product, 145 bases in length, was resolved by PAGE and stained with ethidium bromide [20,45]. The number of PCR cycles was determined within a linear amplification range.

2.6. Western blotting of microsomal proteins

The isolated kidneys and livers were excised quickly, and then cut into small pieces before homogenization with ice-cold 1.15% KCl. Microsomal fractions were prepared after ultracentrifugation of the 10,000 *g* supernatant at 104,000 *g* for 60 min at 4°. Microsomal protein concentration was determined as described [20,44,46] using bovine serum albumin as a standard. Four micrograms of hepatic microsomal protein or twenty micrograms of renal microsomal protein was resolved by 10% SDS–PAGE and then transferred to a Hybond-C membrane. The localized P450 species were detected using rabbit polyclonal antibodies against rat CYP2B1 protein, which cross-reacted with CYP2B9 and CYP2B10, followed by a biotinylated goat anti-rabbit IgG and biotinylated horseradish H-avidin complex, and then visualized with 3,3′-diaminobenzidine and hydrogen peroxide [17,20].

2.7. Electrophoretic mobility-shift assay

C57BL/6NCrj male mice were administered 10 mg/kg/day of Dex or 100 mg/kg/day of PB three times. The mice were decapitated 24 hr after the last injection. The nuclear extracts were prepared from livers and kidneys as described [47]. A double-stranded DNA probe for the NR1 site in the PBREM of the *Cyp2b10* gene was generated by annealing complementary single-stranded oligonucleotide (5′-TCTGTACTTTCCTGACCTTG-3′, 5′-CAAGGTCAGGAAAGTACAGA-3′). DNA (2.5 pmol) was end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase, and unincorporated nucleotides were removed by MicroSpin G-25 columns (Pharmacia Biotech AB). The binding reaction mixture containing 5 μg of crude nuclear extract, 3 μg of poly(dI-dC-dI-dC), 20,000 cpm of probe, and 1x binding buffer was incubated at room temperature for 30 min. For competition, a 100-fold excess of non-labeled probe was added to the incubation mixture. Protein–DNA complexes were resolved by 4.5% nondenaturing PAGE.

3. Results

3.1. Induction of CYP2B expression by glucocorticoid and PB in mouse kidneys and liver

The constitutive expression level of CYP2B mRNAs in kidney was higher in female (4.4-fold) than in male mice as also observed in the mouse liver on northern blots (Fig. 1A).

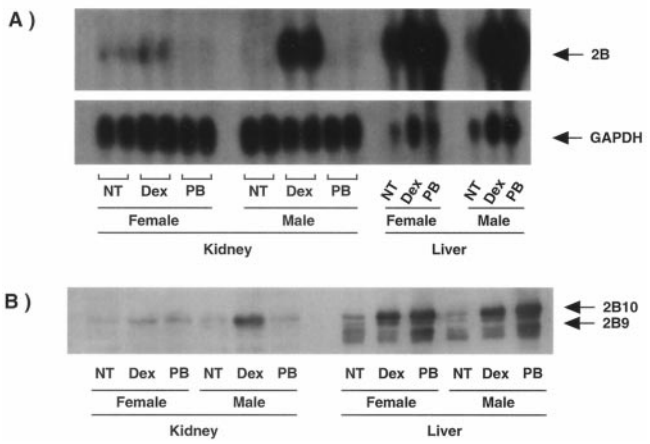

Fig. 1. Expression of CYP2B mRNAs in mouse kidney and liver. Male and female C57BL/6NCrj mice were injected three times with Dex (10 mg/kg/day) and PB (100 mg/kg/day), s.c. and i.p., respectively. The animals were killed 24 hr after the last injection. (A) Northern blot of total RNA from individual animals, using a cDNA probe for the *Cyp2b10* gene. (B) Western blot of renal and hepatic microsomal proteins. Besides CYP2B9 and CYP2B10, an unidentified protein beneath both CYP2B9 and CYP2B10 was present in the liver. Abbreviations: NT, no treatment; Dex, dexamethasone; and PB, phenobarbital.

PB and Dex induced the expression of hepatic CYP2B mRNAs in both sexes to the same extent after normalization with GAPDH as a standard. In contrast, Dex, but not PB, induced the expression of CYP2B mRNAs in kidney to a far greater extent in male (8.0-fold induction) than in female mice. Western blots of microsomal proteins revealed that Dex induced CYP2B10 in both sexes and simultaneously suppressed the constitutively expressed CYP2B9 in the liver of female mice, while PB induced both CYP2B9 and CYP2B10 in both sexes in the liver. In the kidney, induction of CYP2B10 by Dex was prominent in male and female mice, whereas induction by PB was not (Fig. 1B).

3.2. Dose–response effect of glucocorticoid on CYP2B mRNAs and protein expression in mouse kidneys

Dex dose-dependently induced the expression of CYP2B mRNAs in kidneys with a higher level of induction in male than in female mice (Fig. 2A), whereas the dose-dependent induction of these mRNAs in liver differed little between the sexes [20]. Western blotting of the microsomal proteins also revealed that Dex treatment increased renal CYP2B10 protein levels in a dose-dependent manner, with the induction far greater in male than in female mice (Fig. 2B). From the RT–PCR analysis, the dose-dependent induction by Dex was confirmed to be due to the increase of the CYP2B10 species, although the constitutive expression of CYP2B10 was nearly undetectable in male mice, even after 30 cycles of amplification (Fig. 3); the expression of CYP2B9 was considerably suppressed in female mice even at the lowest dosage (3 mg/kg/day) employed in the experiment (data not shown). As observed in our previous report [20], besides

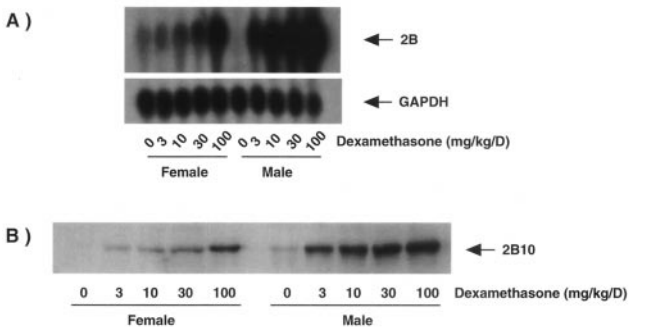

Fig. 2. Dose-dependence of CYP2B expression in kidneys of mice treated with Dex. Male and female C57BL/6NCrj mice were injected subcutaneously three times with the indicated dosage (mg/kg/day) of Dex. The animals were killed 24 hr after the last injection. (A) Northern blot of total RNA, using a cDNA probe for the *Cyp2b10* gene. (B) Western blot of the renal microsomal proteins.

Cyp2b9 and *Cyp2b10*, Dex induced the expression of an unknown species indigestible by either *Bgl*II or *Hha*I. Using a DNA data bank, we compared nucleotide sequences of mouse *CYP2B13*, *CYP2B19*, and *CYP2B20* cDNA [48–50], and considered the possibility of detecting these species with the present RT–PCR method. However, there is no evidence that the unknown species is one of the above species, in which the existence of an *Hha*I site was reported. The characterization of this gene is now in progress.

3.3. Effect of RU-486 on CYP2B induction by glucocorticoid and PB in vivo *and in culture*

Dex- and PB-inducible CYP2B mRNA expression after concomitant treatment with RU-486, a potent anti-glucocorticoid, was examined in both liver and kidney, as well as in cultured hepatocytes. RU-486 suppressed the induction of CYP2B mRNAs by Dex in both organs in

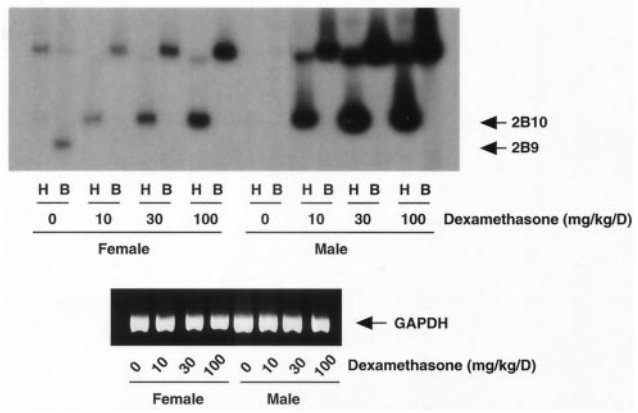

Fig. 3. Discrimination of CYP2B9 and CYP2B10 mRNAs from mouse kidney treated with Dex. Male and female C57BL/6NCrj mice were injected subcutaneously three times with the indicated dosage (mg/kg/day) of Dex. The animals were killed 24 hr after the last injection. One microgram of total RNA was reverse-transcribed and then amplified as described. PCR, 30 cycles; B, *Bgl*II; H, *Hha*I.

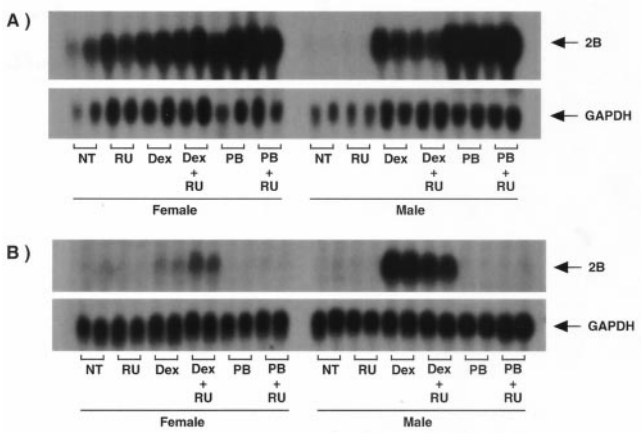

Fig. 4. Effect of RU-486 on Dex- and PB-inducible expression of CYP2B mRNAs *in vivo*. Male and female C57BL/6NCrj mice were injected three times with 10, 10, and 100 mg/kg/day of RU-486, Dex, and PB, respectively. The animals were killed 24 hr after the last injection. (A) Northern blot of total hepatic RNA from individual animals, using a cDNA probe for the *Cyp2b10* gene. (B) Northern blot of total renal RNA from individual animals, using a cDNA probe for the *Cyp2b10* gene. Abbreviations: NT, no treatment; RU, RU-486; Dex, dexamethasone; and PB, phenobarbital.

male mice, while in females Dex slightly increased expression in liver, and doubled expression in kidney (Fig. 4). On the other hand, RU-486 did not affect the PB-inducible expression of CYP2B mRNAs in the liver of either sex. Furthermore, RU-486 alone at the dosage employed in the present experiments induced hepatic CYP2B mRNA expression specifically in females (more than a 3-fold induction), whereas no detectable effect on the expression of these species was observed in the kidney of either sex. In contrast to the *in vivo* results, RU-486 reduced the Dex-inducible expression of CYP2B mRNAs (more than 45%) in cultured hepatocytes of both sexes, but increased PB-inducible expression (Fig. 5). In addition, RU-486 itself induced the expression of these species in hepatocytes cultured from both sexes.

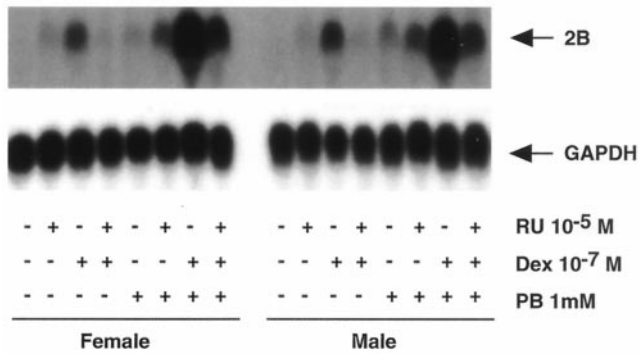

Fig. 5. Effect of RU-486 on Dex- and PB-inducible expression of CYP2B mRNAs in primary cultures. At day 3 hepatocytes were incubated with Dex (10^{-7} M) in DMSO and/or PB (1 mM) in PBS in the presence or absence of RU-486 (10^{-5} M) in DMSO. The control group was left untreated. Total RNA was prepared 24 hr later, and the expression of CYP2B mRNAs was analyzed by northern blotting. Abbreviations: RU, RU-486; Dex, dexamethasone; and PB, phenobarbital.

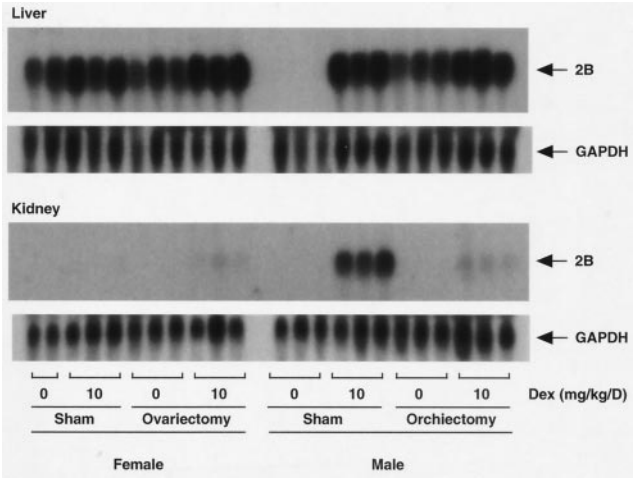

Fig. 6. Expression of Dex-inducible CYP2B mRNAs in gonadectomized mice. Six-week-old C57BL/6NCrj male and female mice were sham-operated or gonadectomized, and 2 weeks after the operation were injected subcutaneously once daily with 10 mg/kg/day of Dex three times. The animals were decapitated 24 hr later. Total RNA was prepared from the liver and kidneys of individual animals and analyzed by northern blotting.

3.4. Effect of gonadectomy and glucocorticoid supplementation on the level of CYP2B expression in mouse kidney and liver

Since the constitutive expression of the *Cyp2b9* and *Cyp2b10* genes differed with sex, gonadectomy was carried out to test whether sex hormones influence the expression of these isoenzymes in kidney and liver. The constitutive expression of CYP2B mRNAs in the male liver was increased markedly, whereas it was not influenced in kidneys of either sex after gonadectomy (Fig. 6). There was no difference in the Dex-inducible CYP2B mRNA expression level between the livers of sham-operated and gonadectomized mice in either sex, whereas it was reduced in the kidney of gonadectomized male mice. The suppressive effect of gonadectomy on the level of Dex-inducible expression of CYP2B mRNAs in kidneys of females was not observable because of the low basal expression.

3.5. Comparison of the induction of CYP2B expression among P450 inducers

The inducibility of CYP2B mRNAs by two other P450 inducers, PCN and DDT, was investigated. In the liver, PCN and DDT markedly increased CYP2B mRNA expression (PCN: female 11.8- and male 21.1-fold induction; DDT: female 15.3- and male 32.5-fold induction, respectively) as did Dex and PB (Fig. 7A), whereas, similar to PB, these inducers were without effect in the kidneys of either sex (Fig. 7B). PCN and DDT, as well as PB, increased both CYP2B9 and CYP2B10 hepatic protein levels, whereas Dex induced both hepatic and renal CYP2B10 and simultaneously suppressed hepatic CYP2B9 (Fig. 7C).

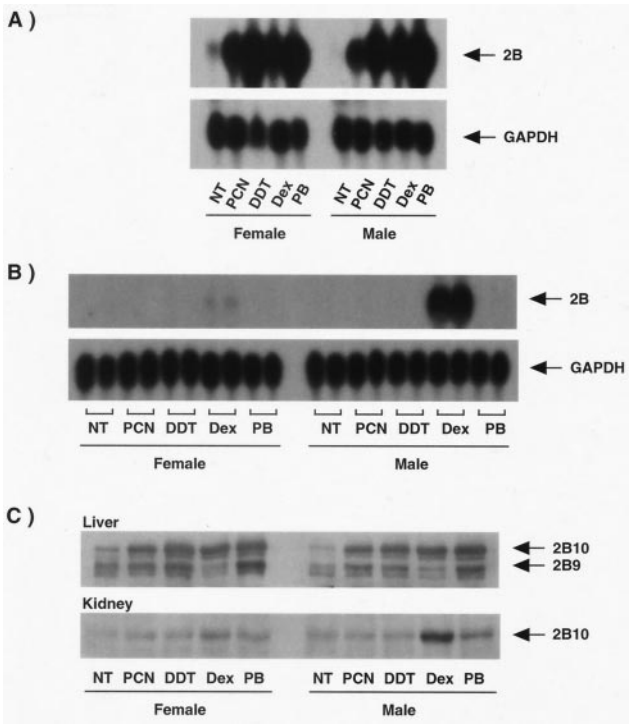

Fig. 7. Effects of various P450 inducers on the expression of CYP2B in mouse liver and kidneys. Male and female C57BL/6NCrj mice were injected with 100 mg/kg/day of PCN, DDT, and PB, or 10 mg/kg/day of Dex once daily three times. The animals were killed 24 hr after the last injection. (A) Northern blot of total hepatic RNA, using a cDNA probe for the *Cyp2b10* gene. (B) Northern blot of total renal RNA from individual animals, using a cDNA probe for the *Cyp2b10* gene. (C) Western blot of the microsomal proteins. Abbreviations: NT, no treatment; PCN, pregnenolone-16α-carbonitrile; DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; Dex, dexamethasone; and PB, phenobarbital.

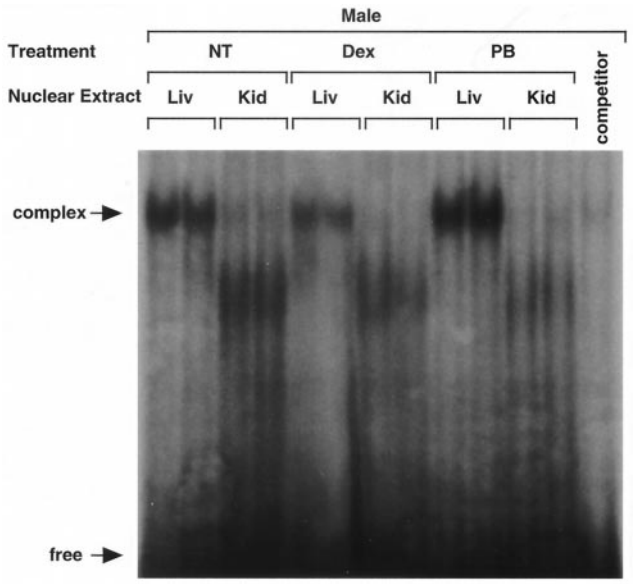

Fig. 8. Electrophoretic mobility-shift assay of CYP2B expression in mouse liver and kidneys. Male C57BL/6NCrj mice were injected three times with Dex (10 mg/kg/day) or PB (100 mg/kg/day). The animals were killed 24 hr after the last injection. Nuclear extracts were prepared from liver and kidneys of individual animals, and the gel-shift assay was carried out as described. Abbreviations: NT, no treatment; Dex, dexamethasone; PB, phenobarbital; Liv, liver; and Kid, kidney.

3.6. Electrophoretic mobility-shift assay

Liver and kidney nuclear extracts were prepared from male mice treated with either PB or Dex. A gel-shift assay was performed with ^{32}P-labeled NR1 oligonucleotides (Fig. 8). NR1 formed one DNA–protein complex with nuclear extract from the livers of mice treated with PB and Dex, as well as with extract from untreated mice, which was eliminated upon incubation with excess unlabeled probe. The amount of DNA–protein complex from nuclear extracts of PB-treated mice was increased, whereas that from Dex-treated mice was decreased. No such complex was observed in the kidney even after treatment with Dex or PB. The same profile was obtained using nuclear extracts from female mice (data not shown).

4. Discussion

The present investigations revealed that Dex dose-dependently induced both hepatic and renal CYP2B mRNA expression in both male and female mice; the level of induction was similar in the liver of both sexes, but was far higher in the male than in the female kidneys. CYP2B10 was predominantly induced by Dex in the kidney. In contrast, PB induced the expression of these two isoforms in the liver but not in the kidney. These observations suggest that the two inducers mediate activation of the *Cyp2b9* and *Cyp2b10* genes through different regulatory pathways and that there are several mechanisms for CYP2B9 and CYP2B10 mRNA expression, the regulatory pathways for which differ between the liver and the kidney.

The difference in PB-inducible expression of *Cyp2b* genes between the liver and kidney is presumably due to the presence of nuclear proteins occupying the NR1 site (Fig. 8), the DNA region most important for PBREM function in both mouse liver and hepatocytes [33]. Among nuclear factors, CAR is considered to be involved in the regulation of PB-inducible *Cyp2b10* gene expression [51]. CAR binds to the NR1 site of the PBREM as a heterodimer with RXR, which serves as a common heterodimerization partner for many orphan nuclear receptors [52]. In principle, two types of mechanisms can explain the activation of CAR DNA-binding by PB [38]. PB may increase CAR DNA-binding activity in liver nuclei, and thereby increase PBREM-dependent gene transcription, by stimulating translocation of CAR from the cytosol to the nucleus. Alternatively, CAR could be constitutively localized in the nucleus, in which case PB would need to activate the DNA-binding activity of the CAR-RXR heterodimer. However, since CAR is a constitutive active receptor that activates the PBREM in the absence of PB exposure [34], the DNA–protein complex of NR1 and the nuclear extract from the untreated mouse liver

have also been observed (Fig. 8). Although several GREs were predicted in the 5′-flanking region of the mouse *Cyp2b10* gene, the nuclear protein(s) of the DNA region important for GRE function has not been identified clearly; thus, we cannot discern at present which factor(s) is involved in Dex-inducible expression of the *Cyp2b10* gene. Consistent with the inhibitory effect of RU-486, which suppressed the induction of CYP2B by Dex, but not by PB *in vivo*, a recent investigation using glucocorticoid receptor knockout mice [53] suggested that modulation of CYP2B expression by Dex is mediated after the hormone binds to the receptor, which is not a PB receptor.

Studies on the regulatory mechanism of P450s such as CYP2A1, CYP2A2, CYP2C11, and CYP2C12, which are involved in the catabolism of sex-related steroids, have revealed that their expression levels change after exposure to androgens or estrogens [54,55]. However, it is not known whether other P450s, the expression of which differs between males and females, are regulated by sex hormones. The constitutive expression of the genes has been shown to exhibit sexual dimorphism, and sex hormones by themselves have a potency to induce CYP2B9 and CYP2B10 expression [17]. In the present experiment, gonadectomy significantly changed the expression of CYP2B mRNA in the liver of male mice and in the kidneys of Dex-treated male mice. This finding implies that sex hormones, especially male sex hormones, participate in the regulation of constitutive and Dex-inducible CYP2B expression in the liver and kidneys of adult mice. Furthermore, our preliminary data indicated that the estrogen-responsive element was located in the area known as the PBREM up to −2.4 kbp in the 5′-flanking region of the *Cyp2b10* gene (unpublished data). Therefore, it is worth investigating the expression of these species in prepubertal gonadectomized mice, after supplementation with sex hormones, to further define the role of these hormones.

RU-486 is a potent antiprogestin that binds to the GR [26,56]. The present observation in cultured hepatocytes, that RU-486 suppressed the induction of CYP2B by Dex, suggested that RU-486 modulates the expression of CYP2B by competitively binding to the same GR as Dex. Binding to the GR might be required for induction of the *Cyp2b* gene by Dex [19]. Interestingly, RU-486 increased PB-inducible expression of CYP2B. Dex itself induced CYP2B and is required for a robust induction of CYP2B by PB [20,53]. These findings suggest that the binding of RU-486 or Dex to GR enhances the PB-inducible pathways by promoting or modulating the ability of the PBREM to respond to the inducer in cultured mouse hepatocytes. Since RU-486 has also been reported to be a mouse PXR activator [26], the finding that RU-486 itself was a female-specific inducer in the liver suggests the involvement of other GRE pathways, such as PXR [26,57]. The different effect of RU-486 compared with Dex on the expression of CYP2B mRNA between sexes raised the possibility that the character of GR responsible for the induction of these genes differs between sexes or interacts with some protein such as a male- or female-specific cofactor. Alternatively, the dose of RU-486 employed in the present experiment could be inadequate to competitively suppress the Dex-inducible effect of CYP2B mRNA in the female. However, the regulatory mechanism concerning RU-486 appears to be complicated and needs to be clarified further since RU-486 at the concentration used in the experiment with cultured hepatocytes effectively activates mouse PXR [26].

In addition, two other compounds that are known to induce CYP2B isoenzymes, PCN and DDT, modulated the expression of CYP2B mRNA with different induction patterns in liver (good induction in both sexes) and kidney (no induction in either sex). Interestingly, the PCN-induced pattern of CYP2B mRNA expression was the same as that for PB-induction, although PCN and Dex are considered to be structurally similar [25]. This suggested that there was no correlation between the chemical structure of the inducer and the regulatory mechanism of CYP2B expression. DDT affected the activation of these genes in the same way as PB: it induced these two isoforms in the liver but not in the kidney. The report of Negishi and coworkers [58] and our unpublished results indicate that the induction of CYP2B by DDT is mediated through PBREM.

Understanding the way in which drugs induce P450s (particularly whether they share identical regulatory mechanisms) is biologically relevant because it ultimately leads to better models for screening and predicting drug interactions that occur due to the induction of individual P450s. Since Dex is widely used as a clinical drug and extensively administered concurrently with medications, it is worth investigating the factors associated with the sexually dimorphic expression of *CYP2B* genes to explain their different responses and to further understand the regulatory mechanism in human orthologous P450s.

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