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Interactions of Gender, Growth Hormone, and Phenobarbital Induction on Murine Cyp2b Expression

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ABSTRACT. The interactions of gender, growth hormone, and phenobarbital induction on Cyp2b expression were examined in phenotypically normal (*lit/+*) and growth-hormone deficient "little" (*lit/lit*) mice. Using an immunocrossreactive monoclonal antibody designed to identify rat CYP2B1 and 2B2 proteins, we observed three hepatic Cyp2b proteins in control (*lit/+*) females, but only two proteins, one at trace levels, in control males. Phenobarbital administration to *lit/+* mice increased the expression of the two Cyp2b isoforms in the males by 3- to 4-fold, but produced an approximately 75% reduction in the female-expressed proteins. Whereas growth hormone depletion (*lit/lit*) had no effect on the expression profile of Cyp2b proteins in females, it had a de-repressive effect in males, resulting in the expression of three proteins at concentrations now comparable to those observed in female liver. Generally, phenobarbital had no inductive effects in the *lit/lit* mice of both sexes. In all groups, transcript levels measured by a CYP2B1 probe were in agreement with the protein findings. In contrast, Cyp2b mRNA identified by an oligonucleotide probe for CYP2B2 were repressed completely by growth hormone in both sexes, and was expressed as a female-predominant transcript in the *lit/lit* mice. In spite of an apparent high degree of sequence homology between the rat *CYP2B* and murine *Cyp2b* gene families, the present findings highlight fundamental differences in their constitutive and gender-dependent expression, growth hormone regulation, and phenobarbital inducibility. BIOCHEM PHARMACOL **56**;9:1251–1258, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. murine Cyp2b; growth hormone regulation of Cyp2b; gender differences in Cyp2b; phenobarbital induction of Cyp2b; murine P450; CYP comparisons in rats and mice

Whereas adult rats exhibit an exaggerated and unique sexual dimorphism in CYP-dependent drug metabolism in which the enzyme activities are generally 3 to 5-fold higher in males [1], a reversed sexual dimorphism occurs in mice in which drug-metabolizing enzyme concentrations are only 40–100% greater, depending upon strain, in females than in males [2]. The magnitude of this smaller, but consistent sexual dimorphism in murine drug metabolism is reflected in findings of a less than 2- to 3-fold gender difference in most sex-dependent androgen hydroxylations and their CYP-dependent isoforms [3–7] (compared with many 10- to 20-fold sex differences in the rat [8, 9]), and by the large number of murine microsomal monooxygenases (almost half) that are sex independent [5]. Moreover, in rats an individual sex-dependent form of hepatic CYP may comprise up to 50% of the total CYP [10, 11] and in humans much of drug metabolism is dependent upon just a few forms of CYP [12], but to date no major constituent murine isoform accounting for more than a small percentage of the total hepatic CYP has been identified [6, 13–16].

Gender differences in drug metabolism in rats and mice, and likely many other species, possibly including humans, are regulated by growth hormone [8, 9, 17]. Both male rats and male mice secrete growth hormone in an episodic pattern characterized by lengthy undetectable interpulse periods (i.e. 2–3 hr); female rats and female mice also secrete growth hormone in pulses, but their enhanced pulse rate results in interpulses with a much shorter duration (i.e. <1 hr) than those observed in males [5, 17–19]. These interpulse periods, when growth hormone levels are extremely low or undetectable, are the actual "signals" in the sexually dimorphic growth hormone profiles that regulate the gender differences in both rat and murine drug metabolism [17, 20, 21]. Thus, exposure to the masculine growth hormone profile results in a concomitant expression of male-predominant and suppression of female-predominant CYP isoforms and their dependent drug-metabolizing enzymes, while exposure to the feminine growth hormone profile results in concomitant expression of the female-predominant and suppression of the male-predominant

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CYP isoforms and their dependent drug-metabolizing enzymes. Accordingly, the opposite sexual dimorphism of rat and murine monooxygenase activities can best be understood by considering that the rat liver contains more male- than female-predominant CYP isoforms that are optimally expressed by growth hormone profiles secreted in males, whereas murine liver probably contains more female-predominant isoforms that are optimally expressed by growth hormone profiles secreted in females [5, 17, 22, 23].

In addition to sexual dimorphisms in the expression and regulation of constituent CYP isoforms, similar growth hormone-dependent gender differences regulating phenobarbital induction of hepatic microsomal CYPs have been reported in the rat. Endogenous growth hormone suppresses phenobarbital induction of rat CYP2B1 and 2B2, and the feminine pattern of continuous-like growth hormone secretion is more suppressive than the masculine episodic profile characterized by comparably lengthy interpulses [24, 25]. In contrast to rat CYP2B1 and 2B2, the two characterized isoforms in the murine CYP2B subfamily, i.e. Cyp2b9 and 2b10, are constitutively expressed, are female dependent, and are only minimally or not at all responsive to phenobarbital induction [26, 27]. In an attempt to identify isoforms in the presumptive 16 plus member Cyp2b murine subfamily [28] with characteristics similar to rat CYP2B1 and 2B2, we examined the effects of gender and growth hormone on phenobarbital induction of murine CYPs using monoclonal anti-rat CYP2B1/2 known to crossreact with murine Cyp2bs and oligonucleotide probes against CYP2B1 and 2B2 shown to detect Cyp2b mRNAs [29–32]. Moreover, instead of using the multi-hormone-deficient hypophysectomized mouse, we used the "little" mouse. With a severe deficiency in pituitary growth hormone releasing hormone receptors, the homozygote (*lit/lit*) has <10% normal plasma growth hormone levels, but exhibits no other hormonal abnormalities [33–35]. In contrast, the heterozygote (*lit/+*) possesses the wild-type phenotype [36].

MATERIALS AND METHODS

Mice were housed in the University of Pennsylvania Laboratory Animal Resources facility, under the supervision of certified Laboratory Animal Medicine veterinarians, and were treated according to a research protocol approved by the University Institutional Animal Care and Use Committee. Homozygous affected "little" mice (C57BL/6J-*lit/lit*) and phenotypically normal heterozygotes (C57BL/6J-*lit/+*) were derived in our animal facility from two breeding pairs purchased from The Jackson Laboratory. At all times, animals were housed on hardwood bedding in plastic cages, with a photoperiod of 12 hr light and 12 hr dark at 20–23°. Water, acidified as a prophylactic against the pathogen *Pseudomonas aeruginosa*, and laboratory chow pellets were supplied *ad lib*. At 9 weeks of age, *lit/+* and *lit/lit* male and female mice were injected daily intraperitoneally with either 2 or 10 mg/kg body weight of phenobarbital sodium (Sigma Chemical Co.) or an equivalent sodium concentration of NaCl diluent, pH 9.1. On the morning following the sixth injection of the barbiturate, the mice were decapitated and livers were quickly removed and perfused with ice-cold saline. Each liver from *lit/+* mice was quickly minced; a portion reserved for mRNA determinations was plunged into liquid nitrogen and subsequently stored at −70°. The remaining minced liver was used for microsomal preparations. Due to their small size (<0.5 g), individual livers from *lit/lit* mice were used for either mRNA or microsomal preparations. Livers were never pooled.

Hepatic microsomes were isolated according to our reported method [20] and assayed by western blotting for the presence of murine Cyp2b crossreactive with anti-rat CYP2B1/2 as previously demonstrated [30, 32, 37]. Ten micrograms of microsomal protein was electrophoresed on 0.75-mm-thick SDS-polyacrylamide gels containing 7.5% polyacrylamide and electroblotted onto nitrocellulose filters. The blots were probed with monoclonal anti-rat CYP2B1/2 mouse IgG (Oxford Biomedical Research). The primary antibody was located with horseradish peroxidase conjugated to anti-mouse IgG and detected with minor modification [38] of an enhanced chemiluminescence kit (Amersham).

Total hepatic RNA was isolated using a single-step guanidinium thiocyanate method [39]. Ten micrograms of RNA was electrophoresed under formaldehyde denaturing conditions on 1.2% agarose containing 0.2 mol/L of formaldehyde and transferred to Gene-Screen nylon membranes (New England Nuclear). The blots were probed with a ^{32}P-labeled CYP2B1 oligonucleotide probe (5′-GGTTGG TAGCCGGTGTGA-3′) [40] using hybridization and high stringency washing conditions as described previously [41]. Northern blots were stripped and then reprobed with a ^{32}P-labeled 2B2 oligonucleotide probe (5′-GGATGGTG GCCTGTGAGA-3′) [40]. Evidence that RNA was equally loaded and transferred was obtained by equivalent intensity of ethidium bromide staining of 18S and 28S rRNA bands [42]. Furthermore, the 18S rRNA oligonucleotide probe was used as a control to verify the consistency and integrity of RNA loading [43].

Hepatic microsomal benzphetamine demethylase activity was measured spectrophotometrically as previously described [44].

Data were subjected to analysis of variance, and differences among pairs of means were determined using "*t*" statistics and the Bonferroni procedure for multiple comparisons.

RESULTS

As expected [36], the body weights of the homozygous *lit/lit* mice were about 50% of those of their heterozygous siblings (*lit/+*), whose body weights, in turn, were no different from those of the wild type (+/+) (not reported).

In agreement with previous reports [25, 45], the monoclonal antibody used in the present investigation identified both rat CYP2B1 and 2B2 proteins (Fig. 1). Since the

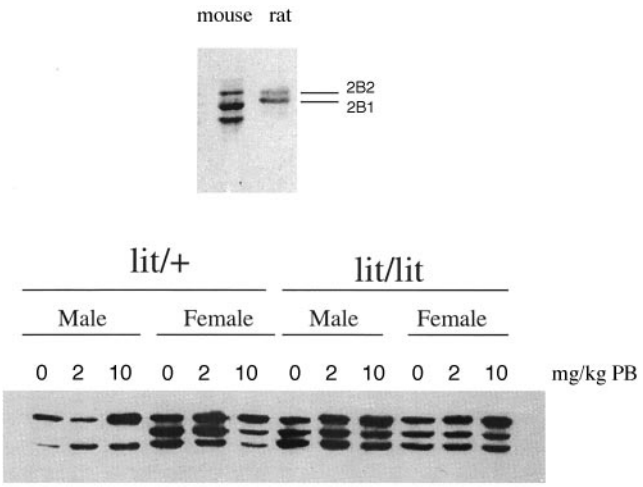

FIG. 1. Hepatic Cyp2b proteins in phenotypically normal (*lit/+*) and growth hormone-deficient (*lit/lit*) male and female mice treated with phenobarbital. Adult mice were injected with either diluent or 2 or 10 mg/kg body weight of the barbiturate for 6 days. Microsomes (10 μg) from at least four different livers for each treatment group were individually analyzed for Cyp2b protein content by western blotting, using an immunoreactive rat CYP2B1/2 monoclonal antibody as described in Materials and Methods. In a control blot (top panel), hepatic microsomes isolated from untreated female CD-1 mice and phenobarbital-treated (10 mg/kg/day for 6 days) female CD rats were probed with the same CYP2B1/2 antibody.

cDNA-deduced amino acid sequences of the three known murine Cyp2b isoforms, i.e. 2b9, 2b10, and 2b13, share an 80–95% sequence similarity with 2B1 and 2B2 [28] and the antibodies are specific in recognizing only members of the 2B family [45], it is not unreasonable to conclude, as have others [30, 32, 37], that the murine proteins identified by anti-rat CYP2B1 and 2B2 are members of the same gene family.

We observed a dramatic sexual dimorphism in the expression levels of murine Cyp2b proteins (Fig. 1). The antibody recognized substantial levels of three putative Cyp2b proteins in the heterozygous *lit/+* female livers, whereas only two proteins, albeit one of them at only trace levels, were detected in the heterozygous *lit/+* male livers. This gender difference in expression of Cyp2b isoforms appears to be under growth hormone control, since the growth hormone-deficient *lit/lit* males expressed the same three proteins, at similar concentrations, as the control females. Growth hormone deficiency had no, or at most a very slight suppressive effect on the levels of the three isoforms of Cyp2b identified in females.

Response to the 2-mg dose of phenobarbital was marginal at best, with the only obvious change being an increase in the concentrations of the lowest molecular weight Cyp2b protein in the *lit/+* males (Fig. 1). The higher 10 mg/kg dose of the barbiturate produced a clear sexual dimorphism in hepatic Cyp2b levels. The concentrations of the two isoforms in the *lit/+* male livers were increased about 3- to 4-fold by the 10-mg phenobarbital dose. In contrast, the two lower molecular weight proteins of Cyp2b in the *lit/+* female livers were reduced to about 25% of normal, while levels of the highest molecular weight isoform remained unchanged. With the exception of a small increase in the concentrations of the higher molecular weight isoforms of Cyp2b in both sexes, phenobarbital had no inductive effect on the cytochromes in either male or female growth hormone-depleted *lit/lit* mice.

In contrast to previous studies that used CYP2B1 or 2B2 cDNA probes to identify murine Cyp2b transcripts [29–31], we used selective 18-mer oligonucleotide probes designed to target the small sequence differences between the isoforms. While the large cDNA probes cannot distinguish between highly homologous mRNAs [40], it is clear from Fig. 2 that the CYP2B1 and 2B2 oligo probes each identified a different murine transcript.

The likelihood that the CYP2B probes could identify any of the Cyp2b murine isoforms (noting that *2b9*, *2b10*, and *2b13* are the only *Cyp2b* genes sequenced [28]) would depend upon the degree of hybridization (i.e. sequence similarity) between the oligo probes and the murine mRNAs. A sequence comparison indicates a 100% match between the CYP2B1 oligonucleotide probe and nucleotide sequences in Cyp2b10 (accession number M21856) and Cyp2b13 (accession number M60357). Similarly, there was a 100% match between nucleotides 2 through 18 of the CYP2B1 probe and nucleotides 36 through 20 of Cyp2b9 (accession number M21855). Consequently, the CYP2B1 probe could have identified any or all of the three murine isoforms. However, irrespective of any possible sequence similarities, it is unlikely that the CYP2B2 oligonucleotide probe had identified Cyp2b9, 2b10, or 2b13. Since the three murine isoforms are all constitutively expressed [4, 26–28, 37, 46] and no transcripts were found in control mice using the CYP2B2 probe, it can be concluded that the CYP2B2-reactive mRNA identified in the *lit/lit* mice had to be an isoform other than Cyp2b9, 2b10, or 2b13.

Using the CYP2B1 oligonucleotide probe, we detected, in agreement with protein levels (Fig. 1), a female-predominant (M:F, 1:3–4) putative Cyp2b murine transcript in *lit/+* male and female mice that responded incrementally to the 2 and 10 mg doses of phenobarbital (Fig. 2A). However, whereas phenobarbital at 10 mg/kg increased the concentration of the CYP2B1-reactive transcript in the male livers by about 3- to 4-fold, it reduced the concentration of the mRNA in the females to about 25% of normal. Growth hormone appeared to suppress expression of the CYP2B1-reactive murine mRNA, since levels were significantly higher in gender-matched control *lit/lit* mice. Again, in agreement with Cyp2b protein findings, 10 mg of phenobarbital increased the Cyp2b mRNA about 4-fold in the *lit/lit* males, but had no effect on transcript levels in the *lit/lit* females.

The CYP2B2 mRNA oligonucleotide probe identified a murine transcript whose expression profile was very different from that detected by the CYP2B1 probe and did not correspond to any of the identified proteins. Irrespective of gender or phenobarbital treatment, no hepatic mRNA was

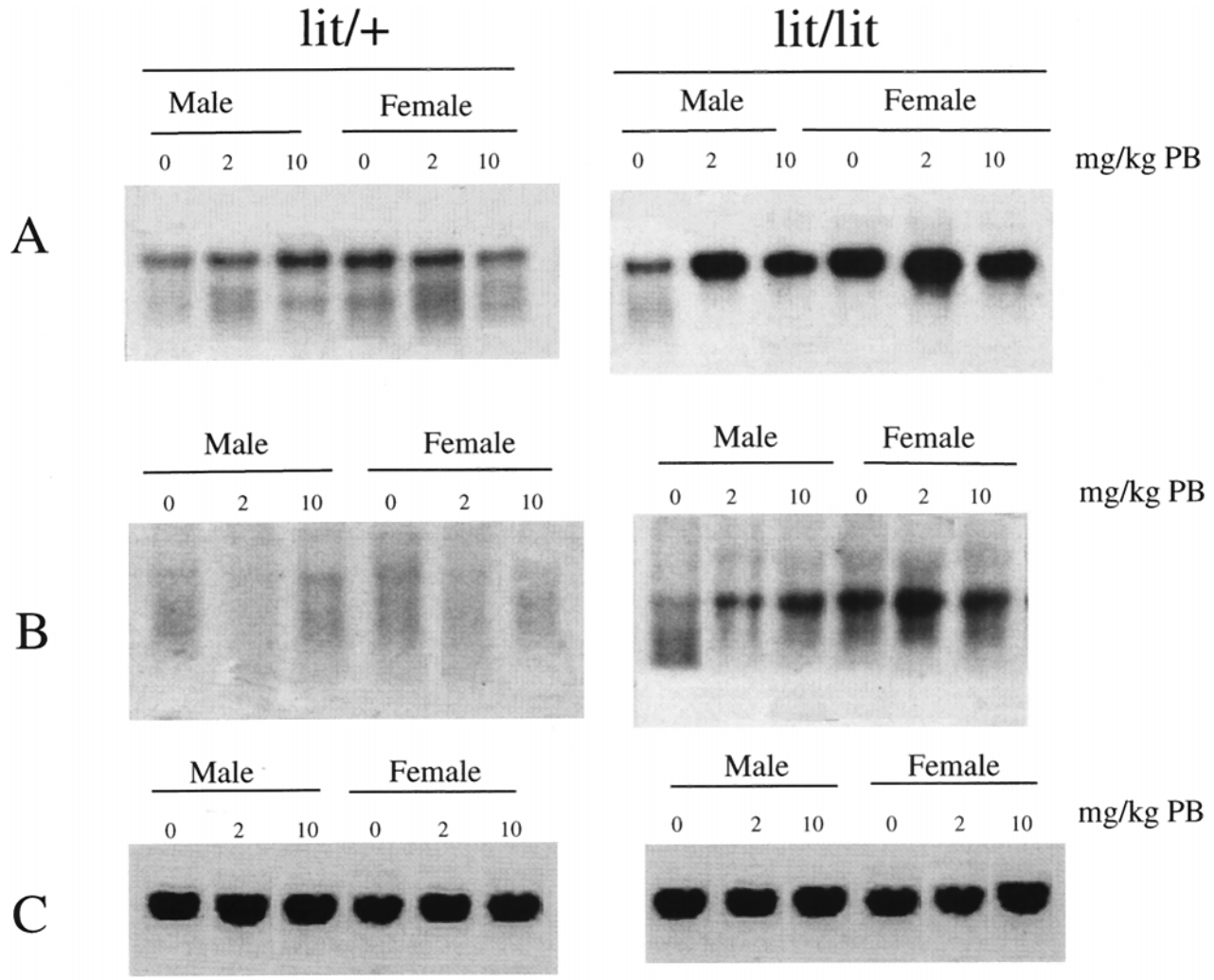

FIG. 2. Hepatic Cyp2b mRNAs in phenotypically normal (*lit/+*) and growth hormone-deficient (*lit/lit*) male and female mice treated with phenobarbital. Adult mice were injected with either diluent or 2 or 10 mg/kg body weight of the barbiturate for 6 days. Total RNA (10 μg) from at least four different livers for each treatment group was individually analyzed for Cyp2b mRNA content by northern blotting, using highly selective 18-mer oligonucleotide probes for rat CYP2B1 (panel A) and rat CYP2B2 (panel B); 18S rRNA (panel C) was used as a control to indicate equal loading of the RNA in all lanes as described in Materials and Methods.

identified in the *lit/+* mice with the CYP2B2 probe. Growth hormone depletion (i.e. the *lit/lit* mice) appeared to de-repress expression of a CYP2B2-related transcript, which was now expressed at 6–7 times the concentration in females as compared with trace levels in males. Phenobarbital administration increased the concentration of the transcript in *lit/lit* males to levels approaching that observed in *lit/lit* females. In contrast, the hepatic mRNA in the *lit/lit* females was basically unresponsive to the barbiturate.

In agreement with our rat studies [47], we found that the use of suboptimal doses of phenobarbital exposed gender differences in the induction of Cyp2b isoforms in mice. The fact, however, that the 2b transcripts and proteins in the female liver were either unaffected or suppressed by the 2 and 10 mg doses of phenobarbital could indicate that the levels of the barbiturate were actually too low to be inductive. Accordingly, we determined the effects of 2 and 10 mg phenobarbital on hepatic microsomal benzphetamine demethylase, a known phenobarbital inducible monooxygenase. We found that the 2-mg dose of the barbiturate induced a small, but statistically significant increase in benzphetamine metabolism in all groups (Table 1). The higher 10-mg dose increased the enzyme activity 2–2.5 times above normal. Considering previous reports of similar inductive levels in benzphetamine demethylase activity in mice treated for 3 days with 80–100 mg/kg body weight of phenobarbital [29, 48], it can be concluded that the suppression or unresponsiveness of Cyp2b isoforms in the female mouse is the normal reaction to clearly inductive levels of the barbiturate.

DISCUSSION

In agreement with earlier reports examining gender dependence and growth hormone regulation of constituent murine CYP isoforms, e.g. Cyp2a, 2c, and 2d [4–7, 13, 17, 22],

TABLE 1. Hepatic microsomal benzphetamine demethylase activity in *lit/+* and *lit/lit* mice treated with phenobarbital

Phenobarbital (mg/kg body weight)	Benzphetamine demethylase activity (nmol/min/mg protein)			
	lit/+		*lit/lit*	
	Male	Female	Male	Female
0	7.4 ± 0.2	6.2 ± 0.3	8.6 ± 0.4*	10.8 ± 0.8*
2	8.7 ± 0.1†	8.9 ± 0.2†	13.1 ± 1.9*†	14.5 ± 1.2*†
10	13.6 ± 1.5†	13.9 ± 2.2†	22.0 ± 0.7*†	25.0 ± 3.9*†

Mice were injected daily with 0, 2, or 10 mg phenobarbital/kg body weight for 6 consecutive days and euthanized on the morning following the last injection. Each value is a mean ± SD, N ≥ 4.

*Significantly different from *lit/+* mice of the same sex and phenobarbital treatment: $P < 0.01$.

†Significantly different from dilutent-treated mice of the same genotype: $P < 0.01$.

we have found that Cyp2b expression is similarly optimized under conditions of growth hormone depletion (e.g. *lit/lit* mouse). Although expression levels of the three Cyp2b proteins in the heterozygous females were basically unaffected by growth hormone, two of the isoforms in the male were suppressed dramatically by the hormone. While expression of the highest molecular weight Cyp2b protein in the heterozygous males (*lit/+*) appeared to be growth hormone independent, two lower molecular weight isoforms, one at trace levels and the other undetectable, became fully expressed in the *lit/lit* males. Cyp2b mRNA results were in agreement with the protein findings. It can be generalized from both previous studies and the present findings that under most circumstances, growth hormone, depending upon its gender-dependent profile, either represses Cyp expression or is simply permissive, having no more effect than when eliminated from the circulation. Thus, in almost every case studied, murine Cyps can be maximally expressed in the absence of growth hormone, which indicates that the hormone functions basically as a Cyp repressor.

The concept that growth hormone may normally regulate hepatic P450 activities by suppressing their expression has some precedence in rat studies. Male-specific CYP2A2 (testosterone 15α-hydroxylase) and CYP3A (testosterone 6β-hydroxylase) levels are optimal in hypophysectomized rats, somewhat suppressed under the influence of the male pattern of growth hormone secretion and fully suppressed when the hormone is secreted in the female profile [9, 49]. However, cytochrome repression is by no means the predominant mechanism of growth hormone action in the rat. In fact, expression of the major isoforms in the rat liver, i.e. male-specific CYP2C11 and female-specific CYP2C12 each comprising up to 50% of the total CYP content in their respective sexes [10, 11], is absolutely dependent upon their homologous growth hormone profiles. In the absence of the hormone, hepatic CYP2C11 levels decline by 80–85% and CYP2C12 levels approach zero [8, 9, 17, 21]. What makes the mouse different is that there appears to be very few isoforms whose expression cannot be maximized by simply eliminating growth hormone.

Due to the unavailability of murine probes to measure Cyp2b, many investigators have conducted mouse studies with probes originally designed to identify rat CYP2B, i.e. highly specific antibodies against CYP2B1 and cDNA probes for CYP2B1 mRNA [29–32, 37]. Although many of our findings are in agreement with these earlier reports (e.g. we have all found higher levels of Cyp2b in females [30, 31]), there is a significant difference in the number of proteins identified. Using untreated control mice, others have reported finding either no hepatic Cyp2b protein in males and one protein in females [31, 32], or one protein in males and two Cyp2b proteins in females [30, 31]. Irrespective of whether the mice were hypophysectomized or treated with phenobarbital, there were no reports of more than two hepatic Cyp2b proteins. Our finding of three proteins can best be explained by our use of a less specific antibody (anti-rat CYP2B1/2) capable of identifying more Cyp2b proteins than anti-rat CYP2B1 [25, 45, 47].

Studies using CYP2B1 cDNA probes have reported higher transcript levels in control females than in control males [30, 31], which is in agreement with both the protein findings and our results using an 18-mer oligonucleotide probe for CYP2B1. Since the cDNA probes are unable to differentiate between CYP2B1 and 2B2 transcripts [40], none of the earlier reports observed the unusual mRNA profile we found with an oligo probe specific for CYP2B2.

In a report limited to male mice [29], hypophysectomy caused a 3- to 4-fold increase in hepatic Cyp2b levels. In another study using both sexes [31], hypophysectomy had a small inductive effect on Cyp2b protein in female liver while inducing a very substantial elevation in males. Comparable results with the *lit/lit* mouse allow us to identify growth hormone as the endogenous pituitary factor normally suppressing Cyp2b expression in male mice. Moreover, since it is the female mouse that expresses the highest concentrations and largest numbers of isoforms, and growth hormone depletion has little effect on their expression, it seems reasonable to conclude that the masculine profile of growth hormone secretion suppresses Cyp2b expression while the feminine pattern of the hormone is permissive, allowing for full expression of the proteins.

Our finding of a several fold increase in Cyp2b proteins and mRNA in *lit/+* male mice treated with phenobarbital is in agreement with other murine studies using rat-designed CYP2B1 probes [29–32]. However, those reports

that included female mice [30, 31] found a similar level of induction in both sexes. For unknown reasons that might include our administration of much lower doses of the barbiturate (~10%) and use of different probes for Cyp2b, we found that phenobarbital produced a dramatic decline in Cyp2b expression in the *lit/+* female. The gender difference in phenobarbital induction was moderated greatly by growth hormone deficiency, with the barbiturate inducing only a modest increase in Cyp2b expression in *lit/lit* males and having no effect in *lit/lit* females.

Although the use of non-murine probes limits our ability to identify the actual isoforms of Cyp2b affected in the study, there are a few reports (almost all from the same group) that have investigated Cyp2b9 and 2b10 expression. In this regard, our largest molecular weight Cyp2b protein (i.e. the top band) and the Cyp2b mRNA identified by the CYP2B1 probe both closely match the characteristics described for Cyp2b10. That is, sharing a 95% sequence similarity with CYP2B1 [26], the molecular weight of Cyp2b10 is slightly greater (i.e. the top band) than that of Cyp2b9 [27]; males express very low concentrations of Cyp2b10 [27, 37] at ~25% of female levels [37]; and phenobarbital administration induces a several fold increase in hepatic Cyp2b10 in males [27, 37, 46]. The sole contradictory finding [27] reports a 15-fold increase in hepatic Cyp2b10 in female DBA/2 and Balb/c mice treated with phenobarbital (100 mg/kg body weight) in comparison with our observation of no induction of the isoform in females treated with phenobarbital (10 mg/kg body weight).

Our intermediate molecular weight Cyp2b protein (having no match with any of the mRNAs in our study) very closely resembles Cyp2b9. Exhibiting just an 80–85% sequence similarity with CYP2B1 [26], Cyp2b9 is only expressed in females [26, 27]; it is uninducible by phenobarbital in either sex, with females showing an apparent barbiturate-induced small decline in Cyp2b9 mRNA [27, 46]; and growth hormone depletion de-represses (i.e. increases) Cyp2b9 mRNA in males to female-like expression levels [4].

Although comparing our observation with other findings in the literature suggests that two of the Cyp2b proteins detected with the rat CYP2B probes are Cyp2b9 and 2b10, the identity of the remaining low molecular weight protein and the mRNA reactive to the CYP2B2 oligonucleotide probe is unknown. Whatever the identity of the CYP2B2-like murine gene, it appears to be totally repressed by both sex-dependent growth hormone secretory profiles and uninducible by phenobarbital in control mice. Eliminating growth hormone (*lit/lit*) results in a sexually dimorphic expression (F > M) in which only the males are now responsive to phenobarbital induction. Since the murine *Cyp2b* gene family contains at least 16 members [28], of which all but two remain virtually uncharacterized, it seems reasonable to conclude that our female-predominant, low molecular weight Cyp2b protein and growth hormone-repressed Cyp2b mRNA are two previously unidentified members of this large supergene family.

In addition to the fact that CYP2B isoforms are not considered constitutive in rats [25, 45], a comparison of the inductive effects of phenobarbital highlights further differences in CYP2B expression in rats and mice. Although induced levels of CYP2B are somewhat higher in male than in female rats [24, 25], they are actually reduced by the barbiturate in female mice. Moreover, whereas phenobarbital induction of CYP2B is maximized in growth hormone-deficient rats of both sexes [24, 25, 45], it has little inductive effect in the comparable mouse model. Irrespective of the actual identities of the Cyp2b isoforms and transcripts detected here, our results demonstrate that, in spite of an apparent high degree of sequence homology between the rat *CYP2B* family and the mouse *Cyp2b* gene families, their constitutive and sexually dimorphic expression, growth hormone regulation, and phenobarbital induction are very different in the two species. This dichotomy is quite similar to differences reported in gender- and tissue-specific expression and catalytic activities of the rat *CYP2C* and mouse *Cyp2c* genes [7]. Why the expression, sexual dimorphism, hormonal regulation, and activities of rat and mouse *CYP* genes in the same family should be so different is curious, but remains speculative.

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