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Biochemical Pharmacology 63 (2002) 2037–2041

Biochemical Pharmacology

Short communication

# Sexual dimorphic expression of mouse hepatic CYP2B: alterations during development or after hypophysectomy

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Received 20 March 2001; accepted 3 December 2001

**Abstract**

The constitutive expression of CYP2B mRNA in the livers of mice in the prepubertal stage was sex-independent, with CYP2B9 as the principal isoform. During the maturation stage, CYP2B10 was expressed in both sexes, whereas CYP2B9 was diminished markedly in the males, resulting in a sexually dimorphic expression in adult mice. Hypophysectomy eliminated the sexual dimorphism in the mouse CYP2B subfamily by markedly increasing the expression of both CYP2B9 and CYP2B10 in males to levels similar to those in females.


*Keywords:* CYP2B; Hypophysectomy; Sex hormones; Development; Sexual dimorphism; Expression

## 1. Introduction

The CYP2B subfamily accounts for one of the prototypic examples of sex-dependent differences in hepatic P450 profiles [1–4]. However, when male and female patterns of hepatic CYP2B expression and metabolism are compared in adult rats and mice, the orientation is opposite: male > female in rats; female > male in mice [2–7]. The sexual dimorphism in rat CYP2B is characterized by temporal patterns of pituitary GH secretion: a characteristic intermittent plasma GH pulse in adult male rats and the continuous presence of plasma GH in adult female rats, resulting in less suppression of CYP2B expression in male than females rats [7–9]. On the contrary, in mice, GH secretion is pulsatile in both males and females, although the interval between pulses is longer in the males [10]; this period of low or no plasma GH is thought to be an important determinant of the male-specific pattern of liver gene expression [11]. Earlier studies have established that pituitary hormones function as the regulator of murine P450s, including the CYP2B subfamily [7,12–14]. However, since earlier studies used rat-derived non-specific cDNA probes or antibodies, it remains to be proven that the sexual dimorphic expression of the major mouse *Cyp2b* genes, namely *Cyp2b9* and *Cyp2b10*, is due to differences in pituitary factors between males and females.

The present study examined sexual dimorphism in mouse CYP2B by investigating the developmental modification of the expression during the maturation stage and by determining the roles of growth and sex hormones on the regulatory mechanism of these P450 enzymes, using an isoform-specific discriminating method.

## 2. Materials and methods

### 2.1. Animals

ddY mice of both sexes were purchased from Sankyo Experimental Animals. Three mice per group were decapitated at various ages (0, 1, and 3 days, and 1, 2, 3, 5, 7, and 13 weeks) to prepare total RNA. C57BL/6CrSlc mice of both sexes were supplied and hypophysectomized or sham-operated by Sankyo Experimental Animals. At 7 weeks of age, the mice were subcutaneously administered 0.2 mg/kg/day of ES in corn oil for 6 days or 5 mg/kg/day of TE in corn oil for 2 weeks and then were decapitated 24 hr after the last injection. Although the vehicle did not change CYP2B expression significantly in previous studies [2–4],

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*Abbreviations:* P450, cytochrome P450; GH, growth hormone; ES, 17β-estradiol benzoate; TE, testosterone propionate; PBREM, phenobarbital-responsive enhancer module; and STAT, signal transducer and activator of transcription.

0006-2952/02/$ – see front matter 
PII: S0006-2952(02)00989-9

the control group for this study also received the vehicle. Livers were excised immediately after the mice were killed to prepare total RNA.

### 2.2. Northern hybridization to P450 probes

Northern blotting and hybridization were performed as described elsewhere [2–4]. Since CYP2B9 and CYP2B10 mRNAs are very similar in both nucleotide sequence and size [1–4], the two cannot be distinguished. Therefore, the mRNA detected on the northern blots is referred to as CYP2B.

### 2.3. RT–PCR

Total RNA was reversed-transcribed, and cDNAs for mouse CYP2B9 and CYP2B10 were amplified and their differential expression was determined as described elsewhere [2–4].

## 3. Results and discussion

During the prepubertal stage, the constitutive expression of hepatic CYP2B mRNA was similar in both sexes (Fig. 1A). The expression was increased in pubertal female mice, whereas that in males gradually declined to nearly undetectable levels after puberty, corresponding with our previous observations in adult mice [2–4], which demonstrated that the constitutive expression of hepatic CYP2B mRNA in female mouse liver was markedly higher than that in males. Since it was not possible to discriminate between CYP2B mRNAs by northern analysis [1–4], RT–PCR was performed to determine whether one or both

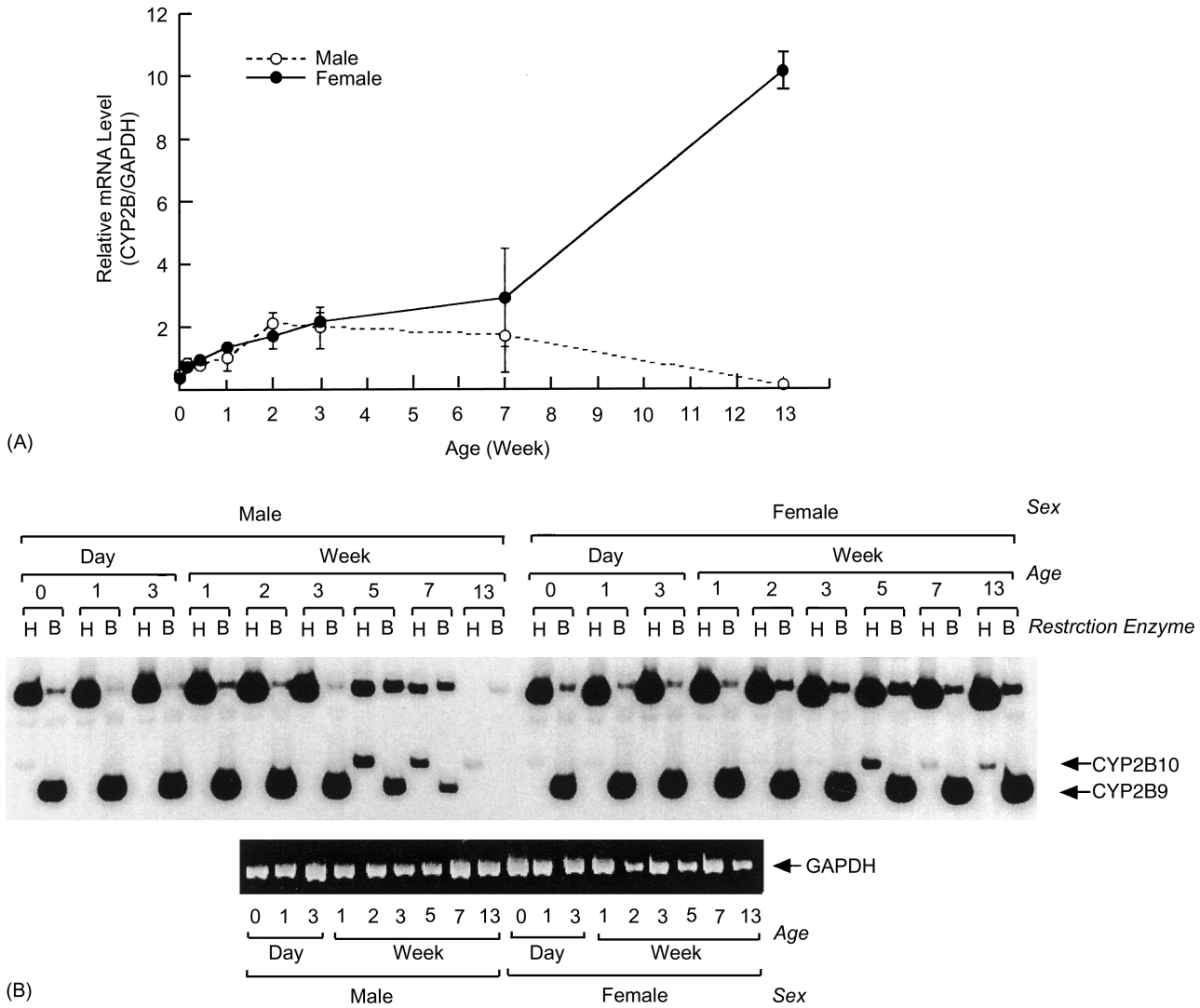


Fig. 1. Developmental profile of CYP2B mRNA expression during maturation. ddY mice of both sexes were killed at the indicated ages. (A) Ten micrograms of total hepatic RNA was northern-blotted and hybridized using a cDNA probe for the *Cyp2b10* gene. The blots were normalized to the signal for GAPDH mRNA. The relative mRNA level was determined densitometrically from autoradiographs and presented with age as a line graph (N = 3: mean ± SD). (B) Discrimination of the developmental expression of CYP2B9 and CYP2B10 mRNA by RT–PCR. Restriction enzymes: H, *Hha*-I; B, *Bgl*-II. A representative experiment of three is shown.

major isoforms, CYP2B9 and CYP2B10, were expressed during the maturation stage. Interestingly, CYP2B9 mRNA was the principal isoform expressed during prepuberty in both sexes (Fig. 1B). The expression of CYP2B10 mRNA was first noted at 5 weeks of age, and it continued throughout the pubertal stage in both sexes. In week 7 CYP2B10 mRNA was reduced slightly in males but markedly in females, whereas over the same time period CYP2B9 decreased sharply in males but not in females. Thus, the ratio of CYP2B9/CYP2B10 mRNA changed noticeably during maturation due to the marked reduction of CYP2B9 mRNA in males after puberty; this observation was consistent with the findings of our previous studies in adult mice [2–4].

Since the expression of CYP2B9 and CYP2B10 mRNA was modified during the maturation stage, we continued our investigation of the effect of sex hormones on the expression of these isoforms in hypophysectomized mice of both sexes. Hypophysectomy markedly increased the expression of CYP2B mRNA in the livers of male mice, in which the levels were usually very low, to a level similar to that in the sham-operated females, while no marked effect was noted in the hypophysectomized females (Fig. 2A). This observation is in accord with earlier reports [7,12–14]. After detection of CYP2B mRNA expression by RT–PCR, it was noted that hypophysectomy more markedly affected the quantities of CYP2B9 than of CYP2B10 mRNA in males. On the other hand, the expression of CYP2B9 mRNA in females was almost unchanged, even after hypophysectomy (Fig. 2B, Table 1). Thus, the ratio of CYP2B9/CYP2B10 mRNA was increased significantly in males, but not in females (Table 1). Treatment of hypophysectomized mice of both sexes with sex hormones reduced the ratio of CYP2B9/CYP2B10 mRNA according to the increase of CYP2B10, except in the case of TE-treated hypophysectomized male mice. This result suggests the possibility that estrogen can regulate *Cyp2b10* gene expression. In our previous experiments [15], we observed that the reporter gene constructs containing a 5′-flanking sequence of the *Cyp2b10* gene could be activated by ES in the absence of growth hormone in mouse primary hepatocyte cultures, and that the activation was mediated via the phenobarbital responsive element PBREM. In accord with our observation, Kawamoto *et al.* [16] reported that estradiol could activate orphan nuclear receptor CAR, resulting in activation of the

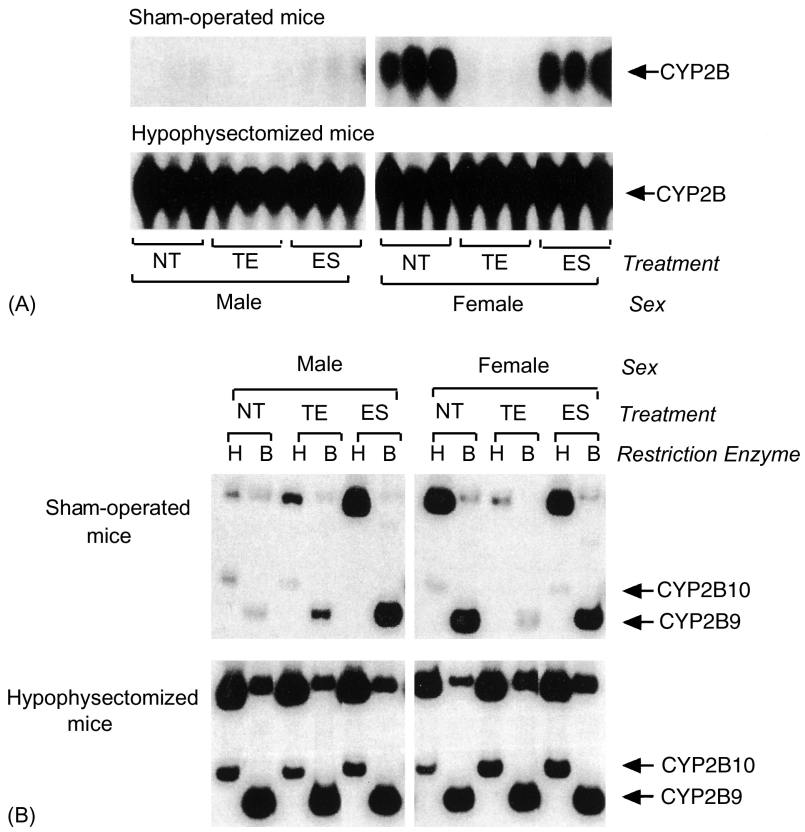


Fig. 2. Modification of CYP2B mRNA expression by hypophysectomy and sex hormone supplementation. 17β-Estradiol benzoate (ES) or testosterone propionate (TE) was administered subcutaneously to hypophysectomized or sham-operated C57BL/6CrSlc mice of both sexes as described in "Section 2"; and the mice were killed 24 hr after the last injection. (A) Ten micrograms of total hepatic RNA was northern-blotted and hybridized using a cDNA probe for the *Cyp2b10* gene. (B) Discrimination of the expression of CYP2B9 and CYP2B10 mRNA by RT–PCR. NT, no treatment. Restriction enzymes: H, *Hha*-I; B, *Bgl*-II. A representative experiment of three is shown.

Table 1
Modification of the ratio of CYP2B9/CYP2B10 expression by hypophysectomy and sex hormone supplementation

| Operation | Treatment | Relative expression[a,b] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Male | | | Female | | |
| | | CYP2B9 | CYP2B10 | Ratio | CYP2B9 | CYP2B10 | Ratio |
| Sham-operation | No treatment | 1.05 ± 1.28 | 0.96 ± 0.66 | 1.09 | 11.89 ± 0.58 | 1.00 ± 0.05 | 11.89 |
| Hypophysectomy | No treatment | 8.03 ± 0.48* | 1.65 ± 0.11 | 4.87 | 10.94 ± 2.08 | 1.61 ± 0.51 | 6.79 |
| | Estradiol | 7.64 ± 0.57 | 2.31 ± 0.75 | 3.31 | 10.54 ± 1.22 | 3.67 ± 0.75** | 2.87 |
| | Testosterone | 7.83 ± 0.51 | 1.53 ± 0.48 | 5.12 | 11.03 | 3.50*** | 3.15 |

Results are expressed as means ± SD or as the mean of radioactivity from 3 or 2 individual mice per group, respectively.
[a] Expression level was quantified by a BAS2000 Image Analyzer after the gel-exposed imaging plate was scanned by a BAS2000 Scanner.
[b] Each signal was normalized by that of GAPDH and then expressed as the relative level of that of CYP2B10 in the sham-operated group of female mice.
* Significantly different from the no-treated sham-operated group ($P < 0.001$, *t*-test).
** Significantly different (by the *t*-test) from the no-treated hypophysectomized group ($P < 0.01$).
*** Significantly different (by the *t*-test) form the no-treated hypophysectomized group ($P < 0.05$).

*Cyp2b10* gene by interaction with the NR1 site in the PBREM.

In accord with the results of a recent study [4], ES stimulated CYP2B9 mRNA expression in intact male mice. However, this stimulation by ES did not occur in hypophysectomized male mice, presumably because CYP2B9 was already maximally up-regulated. In addition, TE did not suppress the expression of CYP2B in hypophysectomized mice of either sex, as observed in non-operated female mice.

Pulsatile GH, but not continuous GH, markedly activates STAT5 in rat liver [17], and the involvement of STAT5b in GH-pulse responsive liver gene expression has been recognized [18,19]. The selective loss of male-specific liver P450 gene expression in STAT5b-deficient male mice is coupled with the expression of P450 genes, which are normally expressed only in females [20]. However, the mechanism responsible for the apparent negative regulation by STAT5b of certain female-expressed liver P450s, such as CYP2B, remains to be elucidated.

The results in this study suggest that, beside glucocorticoid hormones [2,3], the pituitary factor may be one of the key factors in the regulatory pathway of the expression of the CYP2B subfamily in mouse liver. Furthermore, we speculate that the pituitary factor may mediate the suppressive influence on CYP2B expression by TE in male mice.

## Acknowledgments

This work was supported, in part, by Grants-in-Aid from the Japanese Ministry of Education, Culture, Sport, and Sciences, as well as the Smoking Research Foundation.

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