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Hormonal Regulation of Cyp4a Isoforms in Mouse Liver and Kidney

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Abstract

Metabolism of fatty acids and eicosanoids by the CYP4A family is very important in lipid homeostasis and signaling. Mouse Cyp4a subfamily, including Cyp4a10, Cyp4a12a, Cyp4a12b, and Cyp4a14, demonstrate a gender- and strain-specific expression in liver and kidney. In C57BL/6 mouse liver and kidney, Cyp4a12a and 4a12b are male-predominant, whereas Cyp4a14 is female-predominant. Cyp4a10 is female-predominant in liver, but shows no gender difference in kidney. The purpose of the present study was to determine whether sex hormones and/or growth hormone (GH) secretion patterns are responsible for the gender-specific Cyp4a expression in C57BL/6 mice. Gonadectomized mice, GH-releasing hormone receptor-deficient little (lit/lit) mice, and hypophysectomized mice were used with replacement of sex hormones or GH in male or female secretion patterns. Both androgens and male-pattern GH regulated the gender-divergent Cyp4a10, 4a12a, and 4a12b in liver, whereas androgens played an exclusive role in regulating Cyp4a10 and 4a12a in kidney. In contrast, Cyp4a12b was increased by male-pattern GH but not androgens in kidney. The female-predominant Cyp4a14 in both liver and kidney was due to a combined effect of male-pattern GH and androgens. In addition, estrogens played a minor role in regulation of Cyp4a isoforms through an indirect pathway. In conclusion, gender-divergent Cyp4a mRNA expression in liver is caused by male-pattern GH secretion pattern and androgens, whereas in kidney, Cyp4a mRNA expression is primarily regulated by androgens.

Introduction

The cytochrome P450 4A (rodents: Cyp4a; humans: CYP4A) subfamily catalyze the oxidation of a wide variety of substrates, including endogenous lipids and xenobiotics (Okita and Okita, 2001). It is well known that the CYP4A/Cyp4a-catalysed ω -hydroxylation of arachidonic acid to 20-hydroxyeicosatetraenoic acid (20-HETE) plays a critical role in the regulation of renal vascular and tubular functions (Okita et al., 1991; Alonso-Galicia et al., 1999). To date, 2 human CYP4As (4A11 and 4A22), 4 rat Cyp4as (4a1, 4a2, 4a3, and 4a8), and 4 mouse Cyp4as (4a10, 4a12a, 4a12b, and 4a14) have been identified (Hsu et al., 2007). They are predominantly expressed in liver and kidney, and are also detected in intestine, lung, heart, and brain (Okita and Okita, 2001).

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Mouse Cyp4as have shown strain- and gender-specific expression patterns in liver and kidney. Bell et al. (1993) cloned Cyp4a10 and 4a12 from the mouse (CFLP) genomic library, and found mRNA expression of Cyp4a12, but not Cyp4a10, showed a gender-specific expression in both livers and kidneys of CFLP mice with higher levels in males. Jeffery et al. (2004) reported that Cyp4a12 was male-predominantly expressed in livers and kidneys of nine mouse strains. Heng et al. (1997) cloned Cyp4a14 gene from the mouse (C57BL6XCBA) genomic library and showed no gender difference of Cyp4a14 mRNA in livers and kidneys of NMC mice. Clodfelter et al. (2006) found Cyp4a10 and Cyp4a14 mRNA were female-predominant, whereas Cyp4a12 mRNA was male-predominant in livers of 129×BALB/C mice. Recently, Muller et al. (2007) investigated Cyp4a isoforms in kidneys of five mouse strains. Their study demonstrated: 1) Cyp4a10 was female-predominant in kidneys of NMRI, FVB/N, and BALB/C mice, but not in kidneys of 129Sv/J and C57BL/6 mice; 2) Cyp4a12a was male-predominant in all five strains, whereas Cyp4a12b was only detected in kidneys of C57BL/6 without gender difference; and 3) Cyp4a14 was female-predominant in kidneys of the five strains they investigated.

Sex hormones and/or gender dimorphic growth hormone (GH) patterns may contribute to gender-specific expression of mouse Cyp4as. Testosterone markedly induced Cyp4a12 mRNA in kidneys of female NMC mice (Heng et al., 1997). Male 129Sv/J mice that lack Cyp4a14 function had increased blood androgens that led to induction of Cyp4a12 in kidneys, and thus development of hypertension (Holla et al., 2001). Pituitary GH secretion is gender specific in mice, with higher GH levels and longer intervals between pulses in males than females (Holloway et al., 2006). Cyp4a12a mRNA was suppressed in livers of male ICR mice with surgical removal of the pituitary, and was induced after GH treatment (Holloway et al., 2006). Hormonal regulation of hepatic or renal Cyp4a expression can sometimes lead to pathological conditions. For example, androgen treatment increased the Cyp4a-mediated biosynthesis of 20-HETE and caused hypertension in rats (Nakagawa et al., 2003). Therefore, knowledge of the contributions of individual hormones to Cyp4a expression will aid in better understanding and predicting how these enzymes are regulated under normal and pathological hormone levels.

Several mouse models are often used to investigate the effects of hormones on gene expression (Cheng et al., 2006). Gonadectomy (GNX) is the surgical removal of the testes or ovaries, and thus depletes sex hormone production. The *lit/lit* mouse model has a spontaneous mutation in the GH-releasing hormone receptor (GHRH-R) resulting in impaired GH secretion, nonetheless it is still responsive to GH therapy (Beamer and Eicher, 1976; Jansson et al., 1986; Lin et al., 1993; Kasukawa et al., 2003). Hypophysectomy (HX) is the surgical removal of the pituitary and thus disrupts the production of both sex hormones and GH secretion. Due to the availability of congenic strains, easy breeding, robustness, and their importance to human disease models, C57BL/6 mice have been the most widely used inbred mouse strain. In the present study, GNX, *lit/lit*, and HX mice with replacement of sex hormones or GH gender-specific patterns were used to investigate the hormonal regulation of Cyp4a isoforms in livers and kidneys of mice.

Method

Materials

Rat growth hormone (GH) was obtained from Dr. Parlow at the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases (Torrance, CA). Pellets for s.c. release of the hormones used in the study, 5 α -dihydroxytestosterone (DHT), 17 β -estradiol (E2), GH, and placebo, were purchased from Innovative Research of America (Sarasota, FL).

Animals

Age-matched male and female C57BL/6 mice were purchased from Charles River Laboratories Inc. (Wilmington, MA). Mice were housed according to the American Animal Associations Laboratory Animal Care Guidelines and were allowed free access to water and Teklad Rodent Diet #8604 (Harlan Laboratories, Madison, WI). The experimental protocol was approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee (IACUC). Livers and kidneys of mice (n=5/gender/group) were removed at approximately 8 weeks of age, snap-frozen in liquid nitrogen, and stored at -80°C until time of analysis.

Sex Hormone Replacement in Gonadectomized Mice

Mice at 37 days of age were castrated or ovariectomized at Charles River Laboratories. At 54 days of age, the gonadectomized mice were implanted s.c. with DHT (5 mg), E2 (0.5 mg), or vehicle in 21-day-release pellets. Eight groups of mice (n=5/gender/treatment) were included in the present study: 1) naïve male mice (sham-operated male mice treated with placebo), 2) naïve female mice (sham-operated female mice treated with placebo), 3) castration + placebo, 4) ovariectomy + placebo, 5) castration + DHT, 6) castration + E2, 7) ovariectomy + DHT, and 8) ovariectomy + E2. Livers and kidneys were removed at 64 days of age from gonadectomized and age-matched mice.

Growth Hormone Replacement in *lit/lit* Mice

Breeding pairs of mice (C57BL/6J-Ghrhr^{lit}) with heterozygous mutant GH-releasing hormone receptor (GHRH-R) were purchased from the Jackson Laboratory (Bar Harbor, ME) and bred in our laboratory animal facilities. The *lit/lit* mice (dwarf mice with an inactivating mutation of GHRH-R) at 8 to 16 weeks of age were used in the present study, and their respective *lit/+* and *+/+* mice (characterized by normal body size) were used as controls. The mice (n=6/gender/group) were treated with rat GH in male pattern (twice daily, 10 days, i.p. injection, dose of 2.5 mg of GH/day/kg body weight), rat GH in female pattern (continuous infusion via s.c. implanted 1 mg rat GH in 21-day-release pellet), or placebo. Livers and kidneys were removed from mice after treatment.

Hormone Replacement in Hypophysectomized Mice

Mice at 38 days of age were hypophysectomized by Charles River Laboratories. To confirm the surgical effect, hypophysectomized mice that gained weight when given 5% glucose water (w/v) were excluded before the start of study. Mice at 54 days of age (n = 4–6/gender/

group) were treated for 10 days with placebo, 21-day release pellets (containing 5 mg of DHT or 0.5 mg of E2), rat GH in male pattern (twice daily, i.p. injections, dose of 2.5 mg of GH/day/kg body weight), or rat GH in female pattern (continuous infusion via s.c. implanted 21-day-release GH pellet). Placebo-treated mice were used as controls. Livers and kidneys were removed from mice at 64 days of age.

Total RNA Isolation

Total RNA was isolated using RNA Bee reagent (Tel-Test Inc., Friendswood, TX) according to the manufacturer's protocol. RNA pellets were dissolved in diethyl pyrocarbonate-treated deionized water. Total RNA concentrations were quantified spectrophotometrically at 260 nm. Integrity of RNA samples was analyzed by formaldehyde-agarose gel electrophoresis with visualization by ethidium bromide fluorescence under ultraviolet light.

Real Time Polymerase Chain Reaction (RT-PCR)

Real time quantitative PCR by Taqman assay was performed to quantify Cyp4a10, Cyp4a12a, Cyp4a12b, Cyp4a14, and GAPDH mRNA expression according to a previous published method (Gyamfi et al., 2008). Briefly, total RNA (1 µg) was reverse transcribed into cDNA in a total of 50 µl of reaction buffer by adding 2.5 U of Moloney murine leukemia virus reverse transcriptase (M-MLVRT) and 0.66 ng of random hexamers (Invitrogen, Carlsbad, CA). The samples were incubated in a Thermo Cycler at 42°C for 15 min, and followed by 95°C for 15 min. The cDNA thus obtained was diluted 10-fold with water and subjected to real-time PCR. Using Primer Express 2.0 (Applied Biosystems, Foster City, CA), the primers and probes (Table 1) were designed to cross introns to ensure that only cDNA but not genomic DNA was amplified. TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA) was used to prepare a total of 20 µl of the PCR mix, with primers and probes at a final concentration of 909 and 125 nM, respectively. The amplification reactions were performed with an ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, CA) with initial hold steps (50°C for 2 min, followed by 95°C for 10 min) and 40 cycles of a two-step PCR (92°C for 15 s, 60°C for 1 min). The fold changes of mRNA between samples were calculated using the standard CT method. Each sample was measured in triplicate. Each gene was normalized to GAPDH after a validation experiment showing that efficiency of target gene amplification was equal to that of GAPDH.

Statistical Analysis

Data generated from the same gender in control and treated mice were analyzed by one-way analysis of variance, followed by Duncan's post-hoc test. Student's t-test was used to determine differences between genders. Statistical significance was set $P < 0.05$.

Results

The mRNA Expression of Cyp4a Isoforms in Livers and Kidneys of C57BL/6 Mice

Cyp4a mRNA expression in C57BL/6 mice is shown as Figure 1. In liver, Cyp4a12a > 4a10 > 4a12b > 4a14 for males, whereas Cyp4a10 > 4a14 > 4a12a ≈ 4a12b for females. In

kidney, Cyp4a10 > 4a12a > 4a12b > 4a14 for males, whereas Cyp4a10 > 4a14 > 4a12a > 4a12b for females. Cyp4a10 mRNA was about 1-fold higher in female than male livers, whereas it showed no gender difference in kidneys. In both liver and kidney, Cyp4a12a and 12b were predominantly expressed in males, and were almost undetected in females. Cyp4a14 mRNA was about 6-fold higher in female than male livers, and it was almost 25-fold higher in female than male kidneys.

Regulation of Cyp4a mRNA by Sex Hormones in Gonadectomized Mice

As shown in Figure 2A, castration increased Cyp4a10 in male livers, whereas ovariectomy decreased Cyp4a10 in female livers. Androgen (DHT) replacement markedly decreased Cyp4a10 in livers of both male and female GNX mice. Estrogen (E2) replacement decreased Cyp4a10 more in males than in females, resulting in a higher mRNA expression in livers of female ovariectomized mice. GNX had little effect on Cyp4a10 in kidney (Figure 2B). Androgen replacement decreased markedly Cyp4a10 in kidneys of both male and female GNX mice, whereas estrogen replacement had no effect on Cyp4a10 in kidneys.

Castration markedly suppressed Cyp4a12a in male livers, whereas ovariectomy slightly increased it in females (Figure 2C). Cyp4a12a mRNA was markedly increased by androgens and slightly increased by estrogens in livers of both male and female GNX mice. After sex hormones replacement, Cyp4a12a was still higher in male than female livers. Cyp4a12a in kidney was markedly decreased by castration of males, whereas it was slightly increased by ovariectomy of females (Figure 2D). Androgen replacement markedly increased Cyp4a12a in kidneys of both male and female GNX mice. In contrast, estrogens only slightly increased Cyp4a12a in kidneys of male castrated mice.

As shown in Figure 2E, castration markedly suppressed Cyp4a12b in male livers, whereas ovariectomy slightly increased Cyp4a12b in females. Androgens increased Cyp4a12b about 100 fold in male and 50 fold in female livers, whereas estrogens only increased it about 10 fold in male and female livers. Cyp4a12b was higher in male than female livers after androgen replacement, whereas it was not gender different after estrogen replacement. Similar to livers, castration markedly decreased Cyp4a12b in male kidneys, whereas ovariectomy slightly increased Cyp4a12b in female kidneys (Figure 2F). However, androgens and estrogens had little effect on Cyp4a12b in the kidneys of GNX mice.

Castration increased Cyp4a14 mRNA in male livers, whereas ovariectomy markedly decreased it in female livers (Figure 2G). Androgens decreased Cyp4a14 more prominently in male than female livers. In contrast, estrogens slightly decreased Cyp4a14 in male and female livers. As shown in Figure 2H, castration markedly increased Cyp4a14 in male kidneys, whereas ovariectomy had no effect on Cyp4a14 in females. Androgen replacement markedly decreased Cyp4a14 in kidneys of both male and female GNX mice. In contrast, estrogens had little effect on Cyp4a14 mRNA, except that they slightly suppressed Cyp4a14 in kidneys of male castrated mice.

Regulation of Cyp4a mRNA by Growth Hormones in *lit/lit* Mice

To investigate the effects of GH on regulation of mouse Cyp4a, *lit/lit* mice were supplemented with male- or female-pattern GH. As noted in Figure 3A, Cyp4a10 mRNA in livers of *lit/lit* mice was much higher than in wild-type (WT) mice. Male-pattern GH markedly suppressed Cyp4a10 in livers of *lit/lit* mice, and restored it to the same level as that in livers of male WT mice. However, female-pattern GH had no effect on Cyp4a10 mRNA in livers of *lit/lit* mice. Additionally, *lit/lit* mice also had higher mRNA expression of Cyp4a10 in kidneys than did WT mice (Figure 3B). Neither male- nor female-pattern GH had any effect on Cyp4a10 in kidneys of *lit/lit* mice.

The *lit/lit* mice had much lower Cyp4a12a mRNA in their livers than did WT mice (Figure 3C). Male-pattern GH markedly increased Cyp4a12a in both male and female livers, whereas female-GH had no effect. The *lit/lit* mice also had much lower Cyp4a12a mRNA in their kidneys than did WT mice (Figure 3D). Male-pattern GH increased Cyp4a12a in kidneys of both male and female *lit/lit* mice, whereas female-pattern GH had no effect.

Cyp4a12b had a similar regulation pattern as Cyp4a12a in livers of *lit/lit* mice (Figure 3E). Compared to WT mice, *lit/lit* mice had much lower Cyp4a12b mRNA in livers. Male-pattern GH markedly increased Cyp4a12b in livers of both male and female *lit/lit* mice, whereas female-pattern GH had no effect. *Lit/lit* and WT mice had similar levels of Cyp4a12b mRNA in their kidneys (Figure 3F). Male-pattern GH slightly increased Cyp4a12b in kidneys of both male and female *lit/lit* mice, whereas female-pattern GH had no effect.

Cyp4a14 mRNA was higher in livers of *lit/lit* mice than in WT mice (Figure 3G). Male-pattern GH markedly decreased Cyp4a14 in livers of both male and female *lit/lit* mice, whereas female-pattern GH only slightly decreased Cyp4a14. In kidneys, Cyp4a14 was much higher in *lit/lit* mice than in WT mice (Figure 3H). Male-pattern GH decreased Cyp4a14 in kidneys of male but not female *lit/lit* mice, whereas female-pattern GH had no effect on Cyp4a14.

Regulation of Cyp4a mRNA by Hormones in Hypophysectomized Mice

Hypophysectomized (HX) mice were treated with either sex hormones or GH to investigate effects of hormones on Cyp4a mRNA in livers and kidneys. Cyp4a10 mRNA was markedly increased by HX in livers of both male and female mice (Figure 4A). Male-pattern GH markedly decreased Cyp4a10 in livers of both male and female HX mice, whereas female-pattern GH, androgens and estrogens had no effect. Similar to livers, kidney Cyp4a10 was also higher in HX mice than in WT mice (Figure 4B). Androgens markedly decreased Cyp4a10 in kidneys of both male and female HX mice, whereas GH and estrogens had no effect.

After HX, Cyp4a12a mRNA in liver was decreased in males, but increased in females, resulting in similar levels between the two genders (Figure 4C). Male-pattern GH increased Cyp4a12a in livers of male but not female HX mice, whereas female-pattern GH decreased Cyp4a12a in livers of female but not male HX mice. In contrast, androgens and estrogens had little effect on kidney Cyp4a12a in HX mice. As shown in Figure 4D, Cyp4a12a mRNA decreased in male but increased in female kidneys after HX, resulting in similar mRNA

levels between the two genders. Androgens markedly increased Cyp4a12a in kidneys of both male and female HX mice, whereas GH and estrogens had no effect.

Liver Cyp4a12b mRNA was much lower in male HX mice than in male WT mice, whereas it was similar between female HX and WT mice (Figure 4E). Male-pattern GH markedly increased Cyp4a12b in livers of both male and female HX mice, and Cyp4a12b was still higher in livers of male than female HX mice. By contrast, female-pattern GH and sex hormones had no effect on Cyp4a12b mRNA in livers of HX mice. As shown in Figure 4F, kidney Cyp4a12b markedly increased in female, but not in male HX mice. GH and sex hormones had no effect on Cyp4a12b in kidneys of male HX mice, whereas female-pattern GH and estrogens slightly decreased Cyp4a12b in kidneys of female HX mice.

Both male and female HX mice had higher Cyp4a14 mRNA in liver than did WT mice (Figure 4G). Male-pattern GH markedly suppressed Cyp4a14 in livers of both male and female HX mice, whereas female-pattern GH and androgens had no effect. Compared to WT mice, HX mice had much higher Cyp4a14 in their kidneys (Figure 4H). Male-pattern GH decreased Cyp4a14 in kidneys of both male and female HX mice, and restored it to the same level as that of female WT kidneys. However, androgens decreased Cyp4a14 mRNA to almost background level in kidneys of both male and female HX mice.

Discussion

The predominant Cyp4a isoforms in livers and kidneys of C57BL/6 mice are Cyp4a10 and Cyp4a12a for males, and Cyp4a10 and 4a14 for females (Figure 1). Cyp4a10 mRNA in livers of male mice is about half of that in female mice, while it is similar between male and female kidneys. Previous studies showed that Cyp4a10 was minimally expressed and had no gender difference in CFLP mouse livers, whereas Cyp4a10 in livers of male 129XBALB/C mice was about 10% of that in females (Bell et al., 1993; Clodfelter et al., 2006). This suggests that gender-difference in Cyp4a10 is strain-specific. Cyp4a12a and 4a12b are two Cyp4a12 genes that are resulted from a tandem 100kb duplication within the Cyp4abx cluster on mouse chromosome 4 (Nelson et al., 2004). The present study demonstrated that both of them are predominantly expressed in livers and kidneys of C57BL/6 male mice, with extremely low levels in females. In addition, the present study also demonstrated that Cyp4a14 is female-predominant in both livers and kidneys of C57BL/6 mice. The mRNA expression of Cyp4a12 and 4a14 are consistent with previous reports (Clodfelter et al., 2006; Muller et al., 2007).

Cyp4a10 plays an important role in the control of renal Na⁺ transport and ultimately systemic blood pressure. For example, Cyp4a10-null mice developed a salt-sensitive hypertension like most human hypertensions (Nakagawa et al., 2006). In the present study, androgens markedly suppressed both liver and kidney Cyp4a10 in GNX mice (Figure 2), whereas male-pattern GH in *lit/lit* mice only suppressed liver Cyp4a10 (Figure 3). Therefore, liver Cyp4a10 is regulated by stimulatory effects of both androgens and male-pattern GH, whereas kidney Cyp4a10 is affected only by androgens. In hypophysectomized mice, Cyp4a10 was only decreased by male-pattern GH in livers, whereas Cyp4a10 in

kidneys was affected only by androgens (Figure 4). This suggests that male-pattern GH primarily regulates liver Cyp4a10, whereas androgens exclusively regulate kidney Cyp4a10.

Cyp4a12a is the major Cyp4a12 isoform in livers and kidneys of mice, and is the predominant 20-HETE synthase in kidneys of mice (Muller et al., 2007). Cyp4a12a and 4a12b are predominantly expressed in livers and kidneys of male mice, which may explain the gender difference in blood pressure and susceptibility to cardiovascular morbidity (Skott, 2003). Hormonal regulation of Cyp4a12a and 4a12b in mouse liver is complicated. Administration of androgens to GNX mice (Figure 2) and male-pattern GH to *lit/lit* mice (Figure 3) markedly induce Cyp4a12a and 4a12b mRNA in livers. In contrast, sex hormones administered to HX mice have little effect on Cyp4a12a and 4a12b in livers (Figure 4). Male-pattern GH administration to HX mice increases liver Cyp4a12a in males, and liver Cyp4a12b in both males and females. Therefore, Cyp4a12a and 4a12b are male-predominant in liver, mainly due to the stimulation of androgens and male-pattern GH. In addition, male-predominant expression of Cyp4a12a in kidneys is mainly due to androgens (Figure 2F and 4F). Interestingly, male-predominant Cyp4a12b in kidneys is not affected by either sex hormones or gender-specific GH patterns.

Cyp4a14 is inducible by peroxisome proliferators and plays a key role in hepatocyte proliferation, although it is minimally expressed in male mice. Compared to other Cyp4a isoforms, Cyp4a14 has a poor enzyme activity for arachidonic acid and eicosatetraenoic acid metabolism (Muller et al., 2007). Therefore, the hypertension developed in Cyp4a14-null mice is not due to Cyp4a14, but due to increased Cyp4a12 in kidneys (Holla et al., 2001). Unlike other Cyp4a isoforms, Cyp4a14 is suppressed by both androgens and male-pattern GH in livers and kidneys of HX mice, although male-pattern GH has less effect on Cyp4a14 mRNA in kidneys than do androgens (Figure 4H). This indicates that the regulation mechanisms of Cyp4a14 in kidneys are different than those of other Cyp4a isoforms.

Signaling mechanisms other than the classic hormone receptor pathways may also be involved in the regulation of hepatic Cyp4a isoforms. Although estrogens bind primarily to the estrogen receptor, they can activate constitutive androstane receptor (CAR) (Kawamoto et al., 2000). Phenobarbital induces Cyp4a10 and 4a14 in livers of CAR-null mice, indicating that CAR may be a transcription blocker that prevents the induction of these genes (Ueda et al., 2002). In the present study, mouse liver Cyp4a10 and 4a14 are suppressed by estrogens in GNX mice, which may be due to estrogen-mediated CAR activation. Moreover, estrogens slightly induce Cyp4a12a and 4a12b in livers of GNX mice, indicating that CAR may also play a role in maintaining mouse liver Cyp4a12a and 4a12b. Further experiments are required to investigate why and how hepatic Cyp4a10 and 4a14 are suppressed by estrogens.

In summary, all four mouse Cyp4a isoforms are gender-differently expressed in mouse liver, which is due to a combined effect of androgens and male-pattern GH (Figure 5). The hormonal regulation of gender-divergent Cyp4a isoforms in mouse liver and kidney are summarized in Table 2. As indicated from hormone replacements in HX mice, male-pattern GH has the primary role in regulating the gender-different Cyp4a mRNA in livers, whereas androgens have the primary role in kidneys. It has been shown that sex hormones can affect

GH secretion patterns by regulating GH-releasing factor, namely somatostatin, as well as pituitary function (Legraverend et al., 1992; Painson et al., 1992). Therefore, sex hormones, in particular androgens, can influence hepatic gender-divergent Cyp4a by facilitating or strengthening the male-pattern GH in mice (Figure 5).

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Figure 1.

The mRNA expression of Cyp4a10, 4a12a, 4a12b, and 4a14 in livers and kidneys of C57BL/6 mice. Total RNA was isolated and analyzed by real-time PCR for each Cyp4a isoform. The mRNA expression were normalized by GAPDH and are presented as mean $\pm$ S.E.M (n=5 or 6). Asterisk (*) represents statistically significant differences ($P<0.05$) between male and female mice.

Figure 2.

Effects of gonadectomy and sex hormone replacements on the mRNA expression of Cyp4a10, 4a12a, 4a12b, and 4a14 in liver and kidney tissues from control and gonadectomized male and female mice. Total RNA was isolated and analyzed by real-time PCR for each Cyp4a isoform. The mRNA expression were normalized by GAPDH and are presented as mean±S.E.M (n=5 or 6). Different treatment groups are as follows: Naïve (sham-operated male and female mice treated with placebo vehicle), GNX+Plac (vehicle administered to gonadectomized mice), GNX+DHT (5 α -dihydroxytestosterone administrated

to gonadectomized mice), and GNX+E2 (17 β -estradiol administrated to gonadectomized mice). Asterisk (*) represents statistically significant differences ($P<0.05$) between male and female mice; Single dagger (†) represents statistically significant differences ($P<0.05$) between control mice and the same gender, vehicle-treated gonadectomized mice; and double dagger (‡) represents statistically significant differences ($P<0.05$) between vehicle-treated gonadectomized mice and the same gender, gonadectomized mice administered DHT or E2.

Figure 3.

Effects of growth hormone on the mRNA of Cyp4a10, 4a12a, 4a12b, and 4a14 in liver and kidney tissues from control and *lit/lit* male and female mice. Total RNA was isolated and analyzed by real-time PCR for each Cyp4a isoform. The mRNA expression were normalized by GAPDH and are presented as mean±S.E.M (n=5 or 6). Different treatment groups are as follows: Naïve (*lit*/+ or +/+ mice treated with placebo vehicle), *lit*/*lit*+Plac (vehicle administered to *lit*/*lit* mice), *lit*/*lit*+MP-GH (rat GH twice daily administered by i.p. injection to *lit*/*lit* mice mimicking male-pattern GH secretion), and *lit*/*lit*+FP-GH (continuous infusion

to *lit/lit* mice via s.c. implanted 21-day-release 1-mg rat GH pellet mimicking female-pattern GH secretion). Asterisk (*) represents statistically significant differences ($P<0.05$) between male and female mice; Single dagger (†) represents statistically significant differences ($P<0.05$) between control mice and the same gender, vehicle-treated *lit/lit* mice; and double dagger (‡) represents statistically significant differences ($P<0.05$) between vehicle-treated *lit/lit* mice and the same gender *lit/lit* mice following GH replacement treatments.

Figure 4.

Effects of hypophysectomy and hormone replacements on the mRNA of Cyp4a10, 4a12a, 4a12b, and 4a14 in liver and kidney tissues from control and hypophysectomized male and female mice. Total RNA was isolated and analyzed by real-time PCR for each Cyp4a isoform. The mRNA expression were normalized by GAPDH and are presented as mean $\pm$ S.E.M (n=5 or 6). Different treatment groups are as follows: Naïve (sham-operated male and female mice treated with placebo vehicle), HX+Plac (vehicle administered to hypophysectomized mice), HX+MP-GH (rat GH twice daily administered by i.p. injection

to hypophysectomized mice mimicking male-pattern GH secretion), HX+FP-GH (continuous infusion to hypophysectomized mice via s.c. implanted 21-day-release 1-mg rat GH pellet mimicking female-pattern GH secretion), HX+DHT (5 α -dihydroxytestosterone administrated to hypophysectomized mice), and HX+E2 (17 β -estradiol administrated to hypophysectomized mice). Asterisk (*) represents statistically significant differences ($P<0.05$) between male and female mice; Single dagger (†) represents statistically significant differences ($P<0.05$) between control mice and the same gender, vehicle-treated hypophysectomized mice; and double dagger (‡) represents statistically significant differences ($P<0.05$) between vehicle-treated hypophysectomized mice and the same gender hypophysectomized mice following hormone replacement treatments.

Figure 5.

Proposed regulation mechanism of the mRNA of Cyp4a10, 4a12a, 4a12b, and 4a14 in mouse liver and kidney by androgens and male-pattern GH. In liver, male-pattern GH plays the primary role in all four Cyp4a isoforms. In kidney, androgens but not GH directly regulate Cyp4a10 and 4a12a, but not Cyp4a12b. Female-predominant Cyp4a14 in mouse kidney is regulated by both androgens and male-pattern GH.

Table 1

Sequences of primers and probes used for real-time quantitative PCR

Name		Sequence (5'-3')	Accession No.
Cyp4a10	Forward	TCCAGGTTTGCACCAGACTCT	NM010011
	Reverse	TCCTGGCTCCTCTGAGAAG	
	Probe	6-FAM CCCGACACAGCCACTCATTCCTGC BHQ1	
Cyp4a12a	Forward	GCCTTATACGGAAATCATGGC	NM177406
	Reverse	TGGAATCCTGGCCCAACAATC	
	Probe	6-FAM ACTCTGTTCGTGTAATGCTGGATAAATGGGAA BHQ1	
Cyp4a12b	Forward	CCTTATACGGAAATCARGGCAGA	NM172306
	Reverse	TGGAATCCTGGCCCAACAATC	
	Probe	6-FAM TCTGTTCATGTCATGCTGGATAAATGGGAA BHQ1	
Cyp4a14	Forward	CAAGACCCCTCCAGCATTTCC	NM 007822
	Reverse	GAGTCCTTGTCCTTCAGATGGT	
	Probe	6-FAM TGCATGCCCTTCCCACTGGCTTTG BHQ1	
GAPDH	Forward	TGTGTCCGTCTGGATCTGA	NM 001001303
	Reverse	CCTGCTTCACCACTTCTTGA	
	Probe	6-FAM CCGCCTGGAGAAACCTGCCA BHQ1	

Table 2
Summary of gender-divergent regulation of Cyp4a isoforms in mouse liver and kidney

	GNX			Lit/lit			HX		
	Androgens	Estrogens	MP-GH	MP-GH	FP-GH	Androgens	Estrogens	MP-GH	FP-GH
Cyp4a10 (liver)	↓	↓	↓	↓				↓	
Cyp4a10 (kidney)	↓				↓				
Cyp4a12a (liver)	↑	↑	↑	↑				↑ (M)	↓ (F)
Cyp4a12a (kidney)	↑		↑	↑	↑				
Cyp4a12b (liver)	↑	↑	↑	↑				↑	
Cyp4a12b (kidney)			↑	↑			↓ (F)		↓ (F)
Cyp4a14 (liver)	↓	↓	↓	↓	↓			↓	
Cyp4a14 (kidney)	↓				↓			↓	

GNX, gonadectomized mice;
Lit/lit, *lit/lit* mice;
HX, hypophysectomized mice;
Androgens, 5-dihydrotestosterone;
Estrogens, 17β-estradiol;
MP-GH, male-pattern growth hormone;
FP-GH, female-pattern growth hormone;
↑, up-regulation in gene mRNA compared with placebo administration;
↓, down-regulation in gene mRNA compared with placebo administration;