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Endocrine Regulation of Gender-Divergent Mouse Organic Anion-Transporting Polypeptide (Oatp) Expression

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ABSTRACT

Several examples of gender-divergent pharmacokinetics exist in humans and experimental animals, and one reason for these variations may be gender differences in transporter expression. Organic anion transporting polypeptides (Oatp) are transporters involved in hepatic and renal uptake of many organic compounds. In mouse livers, Oatp1a1 is male-predominant, whereas Oatp1a4 is female-predominant. However, in kidneys, Oatp1a1 and Oatp3a1 are both female-predominant. 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 Oatp expression in 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. Androgens increased Oatp1a1 mRNA in liver and kidney, whereas male-pattern GH administration increased Oatp1a1 mRNA in livers but not in kidneys. Hepatic Oatp1a4 mRNA levels were decreased by both androgens and male-pattern GH administration. In kidneys, Oatp3a1 mRNA expression was only induced by androgen treatment. In conclusion, gender-divergent Oatp expression in liver is caused by male-pattern GH secretion pattern and androgens. In kidney, gender-divergent Oatp expression is exclusively caused by stimulation by androgens.

Several examples of gender-dimorphic excretion of organic compounds have been documented in humans and experimental animals. For example, the urinary excretion of chemicals, such as clentiazem, taurocholate, torsemide, dibromosulfophthalein, and nilvadipine metabolites, is less in male than female rats (Tanaka et al., 1991; Nakamura et al., 1993; Terashita et al., 1994; Sato et al., 2000; Kato et al., 2002). In addition, intrahepatic cholestasis is observed in some women during pregnancy (Laatikainen, 1975). The etiology of intrahepatic cholestasis of pregnancy is not clear; however, alteration of transporters, including the organic anion transporting polypeptides (Oatp), seems to be involved (Gartung and Matern, 1997; Vore et al., 1997; Pauli-Magnus and Meier, 2005).

Gender differences in the abundance of membrane transporters can manifest as physiological/toxicological phenomena. For example, rat and mouse Oatp1a1 (Isern et al., 2001; Gotoh et al., 2002) and Oatp3a1 (Melia et al., 1998) are markedly male-predominant. Oatp1a1, which reabsorbs organic anions from the renal tubular lumen, is expressed less in female than male rat kidney and may be responsible for the 250-fold higher urinary excretion rate of exogenously administered estradiol-17β-D-glucuronide in female than male rats (Gotoh et al., 2002). Likewise, organic anion transporter 2 is markedly female-predominant (Buist et al., 2002; Buist and Klaassen, 2004) and correlates with a 70-fold higher urinary excretion of perfluorooctanoic acid in females (Kudo et al., 2002). Conversely, organic cation transporter 2 (MacLeod et al., 1991; Urakami et al., 1999) is male-predominant and leads to a higher urinary secretion of tetraethylammonium in male rats. Several other gender differences in transporter expression have been observed, such as multidrug resistance-associated protein 4 (Chen and Klaassen, 2004; Tanaka et al., 2005) and sodium taurocholate cotransporting polypeptide (Simon et al., 2004), but the implications of such differences are currently unknown.

Gender differences in transporter gene expression may be the result of regulation by sex hormones and/or gender-dimorphic growth hormone (GH) secretory patterns. Androgens and estrogens alter gene expression by directly stimulating gene transcription or stabilizing the mRNA of certain genes (Beato, 1989; Paul et al., 1990; Kimura et al., 1994). GH is also an important regulator of gender-divergent gene

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ABBREVIATIONS: Oatp, organic anion transporting polypeptide(s); GH, growth hormone; HX, hypophysectomy; GHRH-R, growth hormone releasing hormone receptor; DHT, 5α-dihydrotestosterone; E2, 17β-estradiol; bDNA, branched DNA; RLU, relative light unit(s); CAR, constitutive androstane receptor; PXR, pregnane X receptor.

expression. Gender-divergent secretion patterns of GH lead to differential effects on gene expression. In rats, males secrete GH in high-amplitude pulses with a regular frequency. Between pulses, serum GH levels are nondetectable (Tannenbaum and Martin, 1976). In contrast, female rats secrete GH in low-amplitude pulses with greater frequency and higher trough levels than males, resulting in a continuously detectable baseline of serum GH (Saunders et al., 1976). These GH secretory patterns are responsible for male-specific expression of rat Cyp2c11 and female-specific Cyp2c12, respectively (Waxman et al., 1991). GH secretion pattern in male mice is similar to that in male rats (MacLeod et al., 1991), and likewise in female mice, GH is secreted at regular intervals with a nondetectable baseline between pulses; however, the pulses are more frequent (1–1.5 h) than those in male mice (2.5 h) (MacLeod et al., 1991). The GH secretory pattern in male mice is responsible for induction of male-predominant Cyp2D9 and repression of female-predominant Cyp2A4 in liver (Noshiro and Negishi, 1986; Aida and Negishi, 1993).

Several animal models are often used to investigate the effects of hormones on gene expression. Gonadectomy is the surgical removal of the testes or the ovaries, the organs primarily responsible for sex hormone production. Hypophysectomy (HX) is surgical removal of the pituitary, which obliterates the production of several hormones, including luteinizing hormone, follicle-stimulating hormone, adrenocorticotropic hormone, and prolactin. A mutant mouse model, the *lit/lit* mouse, has a spontaneous mutation in the GH-releasing hormone receptor (GHRH-R), which leads to impaired GH secretion (Beamer and Eicher, 1976; Cheng et al., 1983; Jansson et al., 1986; Lin et al., 1993). Unlike HX, the *lit/lit* mouse model circumvents the loss of other pituitary hormones and is still responsive to GH therapy (Noshiro and Negishi, 1986; Kasukawa et al., 2003).

Oatp/OATP are solute carriers that transport a wide spectrum of amphipathic substrates. Oatp are responsible for hepatic uptake and have a partially overlapping, partially distinct set of substrate preferences for organic solutes, such as bile acids, steroid conjugates, and many xenobiotics (Hagenbuch and Meier, 2003, 2004). Renal Oatp, such as Oatp1a1, are apically expressed in the S3 segment of the proximal tubule (Bergwerk et al., 1996). Renal Oatp1a1 is responsible for reabsorption of some organic compounds that are filtered, such as estradiol-17β-glucuronide (Gotoh et al., 2002).

Information on hormonal regulation of Oatp is limited. Oatp1a1 and Oatp3a1 in rats and Oatp1a1 in mice are male-predominant genes that are androgen-dependent (Lu et al., 1996; Melia et al., 1998; Isern et al., 2001). Furthermore, rat Oatp1b2 and human OATP1B3 are signal transducer and activator of transcription 5-regulated genes, and signal transducer and activator of transcription 5 is a transcription factor that is transcriptionally regulated by GH secretory patterns (Wood et al., 2005).

As reported previously, gender differences exist in mRNA expression of hepatic Oatp1a1 and Oatp1a4, as well as renal Oatp1a1 and Oatp3a1 in mice (Cheng et al., 2005a). Both hepatic and renal Oatp1a1 is male-predominant, with 2.2- and 19-fold higher expression in liver and kidney of male than female mice, respectively. Conversely, Oatp1a4 mRNA in female livers is 2.4-fold higher than in males. Renal Oatp3a1 is also male-predominant, with 2.8-fold higher expression in males. Androgen-dependent regulation of mouse Oatp1a1 is the only example that exists in the literature of hormonal regulation of mouse Oatp in liver or kidney (Isern et al., 2001). Therefore, the present study was conducted to determine whether gender-divergent Oatp expression in mouse livers and kidneys is caused by sex hormones and/or GH secretion patterns.

Materials and Methods

Materials. Sodium chloride, HEPES sodium salt, HEPES free acid, lithium lauryl sulfate, EDTA, and D-(+)-glucose were purchased from Sigma-Aldrich (St. Louis, MO). Micro-O-protect was purchased from Roche Bioscience (Indianapolis, IN). Formaldehyde, 4-morpholinepropanesulfonic acid, sodium citrate, and $NaHCO_3$ were purchased from Fischer Scientific (Fairlawn, NJ). Chloroform, agarose, and ethidium bromide were purchased from AMRESCO Inc. (Solon, OH). Rat growth hormone was obtained through 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 this study, 5α-dihydroxytestosterone (DHT), 17β-estradiol (E2), GH, and placebo, were purchased from Innovative Research of America (Sarasota, FL).

Animals. Adult male and female C57BL/6 mice were purchased from Charles River Laboratories Inc. (Wilmington, MA). Mice were maintained in a 12-h dark/light cycle, temperature- and humidity-controlled environment according to the American Animal Associations Laboratory Animal Care Guidelines and were allowed free access to water and rodent chow (Teklad; Harlan, Indianapolis, IN). Livers and kidneys of mice were removed at approximately 8 weeks of age (n = 5/gender), snap-frozen in liquid nitrogen, and stored at −80°C.

Sex Hormone Replacement in Gonadectomized Mice. Mice were castrated or ovariectomized at 37 days of age by Charles River Laboratories. At 54 days of age, DHT (5 mg), E2 (0.5 mg), or vehicle in 21-day-release pellets (Innovative Research of America) were implanted s.c. in the gonadectomized male and female mice. The mice were separated into six treatment groups (n = 5/gender/treatment): 1) castration + placebo, 2) castration + DHT, 3) castration + E2, 4) ovariectomy + placebo, 5) ovariectomy + DHT, and 6) ovariectomy + E2. Placebo-treated, age-matched mice were used as controls. Livers and kidneys were removed at 64 days of age from gonadectomized and age-matched control mice.

GH Replacement of *lit/lit* Mice. Breeding pairs of GHRH-R mutant heterozygous mice (C57BL/6J-Ghrhr^{lit}) were purchased from The Jackson Laboratory (Bar Harbor, ME). After breeding in our laboratory animal facilities, *lit/lit* mice at 8 to 16 weeks of age (dwarf mice with an inactivating mutation of GHRH-R) were used in this study. Their respective *lit*/+ and +/+ mice (characterized by normal body size) were used as controls. The mice (n = 6) were treated for 10 days with rat GH in male pattern (twice daily, i.p. injection, dose of 2.5 mg of GH/day/kg b.wt.), female pattern (continuous infusion via s.c. implanted 21-day-release 1-mg rat GH pellet), and placebo. After treatments, livers and kidneys were removed for total RNA isolation.

Hormone Replacement Treatment of Hypophysectomized Mice. Mice were hypophysectomized at 38 days of age by Charles River Laboratories. Hypophysectomized mice received 5% glucose water (w/v) ad libitum. Hypophysectomized mice that gained weight before the start of the study were excluded under the assumption that their surgery was incomplete. At 54 days of age, the mice (n = 4–6/gender/treatment) 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 b.wt.), or rat GH in female pattern (continuous infusion via s.c. implanted 21-day-release rat GH pellet). Placebo-treated, age-

matched mice were used as controls. Livers and kidneys were removed at 64 days of age for total RNA isolation.

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 resuspended 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.

Development of Specific Oligonucleotide Probe Sets for Branched DNA Analysis. Gene sequences of interest were accessed from GenBank. Probe set design for each mouse Oatp gene has been described previously (Cheng et al., 2005a). Probes were synthesized by QIAGEN Operon (Alameda, CA).

Branched DNA Assay. Reagents required for RNA analysis (i.e., lysis buffer, amplifier/label probe buffer, and substrate solution) were supplied in the Quantigene branched DNA (bDNA) signal amplification kit (Bayer Diagnostics, East Walpole, MA). Each Oatp mRNA level was analyzed according to the previously reported method (Hartley and Klaassen, 2000). Data are presented as relative light units (RLU) per 10 μg of total RNA.

Statistical Analysis. Data were analyzed by one-way analysis of variance, followed by Duncan's post hoc test. Statistical significance was set at $P < 0.05$. If only differences between genders were of interest, data were analyzed by Student's t test, and statistical significance was considered at $P < 0.05$.

Results

Regulation of Mouse Oatp by Sex Hormones. Oatp1a1 mRNA is 1.5-fold higher in male than female mouse liver (Fig. 1A). After surgical gonadectomy, Oatp1a1 mRNA decreased in both male and female liver. Androgen (DHT) replacement markedly increased Oatp1a1 mRNA, but estrogen (E2) replacement had no effects on Oatp1a1 mRNA expression.

Oatp1a1 mRNA expression is also much higher in male than female mouse kidney (Fig. 1B). Castration of male mice decreased Oatp1a1 mRNA, but ovariectomy had no effect. Androgen administered to gonadectomized mice markedly increased Oatp1a1 mRNA level in kidney, but estrogen administration had no effect.

Oatp1a4 mRNA expression is female-predominant in mouse liver, being 3-fold higher in female than male liver (Fig. 1C). Castration increased Oatp1a4 mRNA level in liver, but ovariectomy had no effect. Androgen administration to gonadectomized mice decreased Oatp1a4 expression in both male and female liver. Estrogen treatment of gonadectomized mice decreased Oatp1a4 mRNA level in female mice but not in male mice.

Regulation of Oatp3a1 in kidney is similar to that for renal Oatp1a1. Kidney Oatp3a1 expression is 2.8-fold higher in male than female mice (Fig. 1D). Castration decreased Oatp3a1 mRNA in male mice. Ovariectomy did not alter Oatp3a1 mRNA expression. Gonadectomy abolished the gender difference in Oatp3a1 mRNA expression. Androgen replacement increased Oatp3a1 mRNA abundance in both male and female gonadectomized mice, whereas estrogen replacement had no effect.

Regulation of Mouse Oatp by Hormones in Hypophysectomized Mice. Hypophysectomized mice were treated with sex hormones and GH to determine the effects of each hormone on the expression of Oatp (Fig. 2). In hypophysectomized mice, Oatp1a1 mRNA abundance was decreased to nondetectable levels in livers of both male and female mice (Fig. 2A). Male-pattern GH replacement in hypophysectomized mice increased hepatic Oatp1a1 mRNA expression in both male and female mice, whereas female-pattern GH administration had no effect. Both androgen and estrogen replacement did not increase hepatic Oatp1a1 transcripts in mice after hypophysectomy (Fig. 2A). In contrast, androgen replacement markedly increased hepatic Oatp1a1 mRNA in the gonadectomized mice (Fig. 1A).

Oatp1a1 mRNA expression in mouse kidneys is also male-predominant in control mice (Fig. 2B). In hypophysectomized mice, Oatp1a1 transcripts decreased to background values in both male and female mouse kidneys. Similar to gonadectomized mice, androgen replacement markedly increased renal

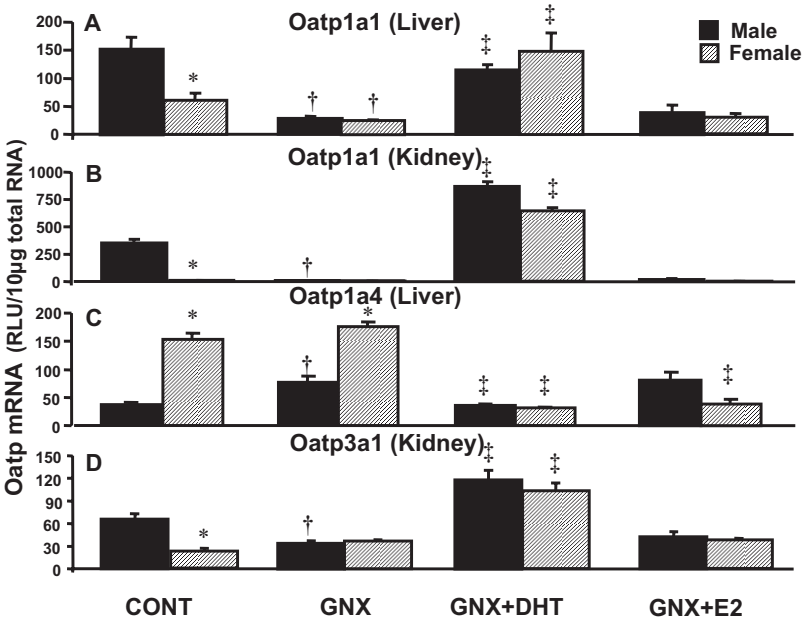

Fig. 1. Effects of gonadectomy and sex hormone replacements on mouse Oatp1a1, 1a4, and 3a1 mRNA expression in liver and kidney tissues from control and gonadectomized male and female mice. Total liver and kidney RNA was isolated and analyzed by the bDNA signal amplification assay for each Oatp mRNA content. The data are presented as mean RLU ± S.E.M. ($n = 5$). GNX (vehicle administered to gonadectomized mice), GNX + DHT (5α-dihydroxytestosterone administered to gonadectomized mice), and GNX + E2 (17β-estradiol administered 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.

Oatp1a1 mRNA in both hypophysectomized male and female mice. GH and estrogen replacement had no effects.

Hepatic Oatp1a4 mRNA abundance is higher in female than male mice (Fig. 2C). Hypophysectomy increased Oatp1a4 mRNA levels in livers of both male and female mice. In hypophysectomized mice, male-pattern GH replacement decreased hepatic Oatp1a4 mRNA levels in both male and female mice, whereas female-pattern GH replacement had no effect. Androgen replacement to hypophysectomized mice did not alter hepatic Oatp1a4 mRNA expression. In contrast, androgens decreased hepatic Oatp1a4 mRNA expression in gonadectomized mice (Fig. 1C). Estrogen replacement increased Oatp1a4 mRNA level in liver of male and female HX mice (Fig. 2C).

Oatp3a1 mRNA expression in mouse kidney is male-predominant (Fig. 2D). In hypophysectomized mice, Oatp3a1 mRNA decreased in male but not in female mouse kidney. Androgen replacement to hypophysectomized mice increased renal Oatp3a1 mRNA, whereas GH and estrogen did not alter renal Oatp3a1 mRNA.

Regulation of Mouse Oatp by Growth Hormone in *lit/lit* Mice. To specifically investigate the effects of GH secretory patterns on the regulation of mouse Oatp, *lit/lit* mice were used, and GH was replaced in a male-pattern or female-pattern secretion. As noted previously, hepatic Oatp1a1 mRNA expression is male-predominant (Figs. 1A and 2A). Oatp1a1 mRNA expression in *lit/lit* mice is much less than in either male or female wild-type mouse livers (Fig. 3A). Male-pattern GH replacement in *lit/lit* mice increased hepatic Oatp1a1 mRNA levels; however, such increases had a very small impact in restoring the expression levels to control values because the increases were relatively small. Female-pattern GH replacement to *lit/lit* mice did not alter Oatp1a1 mRNA expression.

Oatp1a1 mRNA expression in mouse kidney is much higher in male than female mice. In *lit/lit* mice, renal

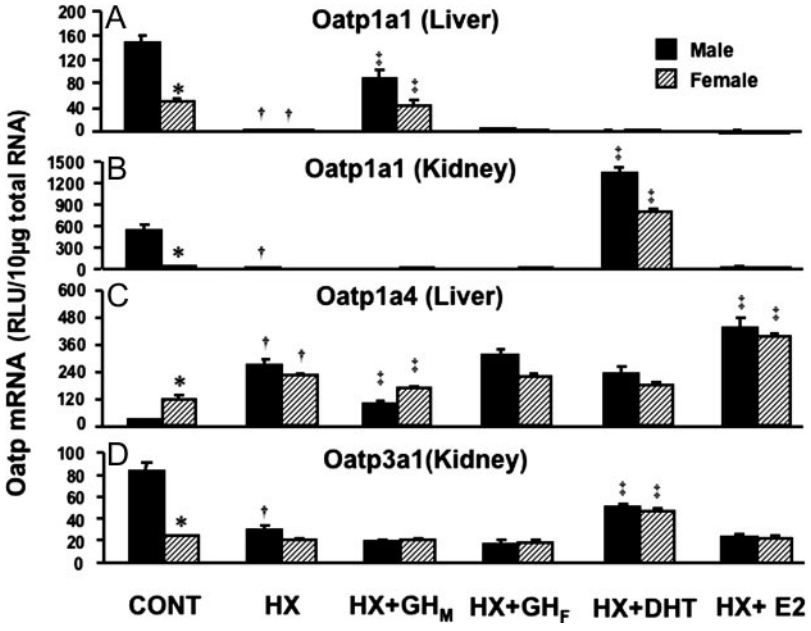

Fig. 2. Effects of hypophysectomy and hormone replacements on mouse Oatp1a1, Oatp1a4, and Oatp3a1 mRNA expression in liver and kidney tissues from control and hypophysectomized male and female mice. Total liver or kidney RNA was isolated and analyzed by the bDNA signal amplification assay for each Oatp mRNA content. The data are presented as mean RLU ± S.E.M. ($n = 5$). HX (placebo administered to hypophysectomized mice), HX + GH_M (rat GH twice daily administered by i.p. injection to hypophysectomized mice mimicking male-pattern GH secretion), HX + GH_F (continuous infusion to hypophysectomized mice via s.c. implanted 21-day-release 1-mg rat GH pellet mimicking female-pattern GH secretion), HX + DHT (5α-dihydroxytestosterone administered to hypophysectomized mice), and HX + E2 (17β-estradiol administered 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.

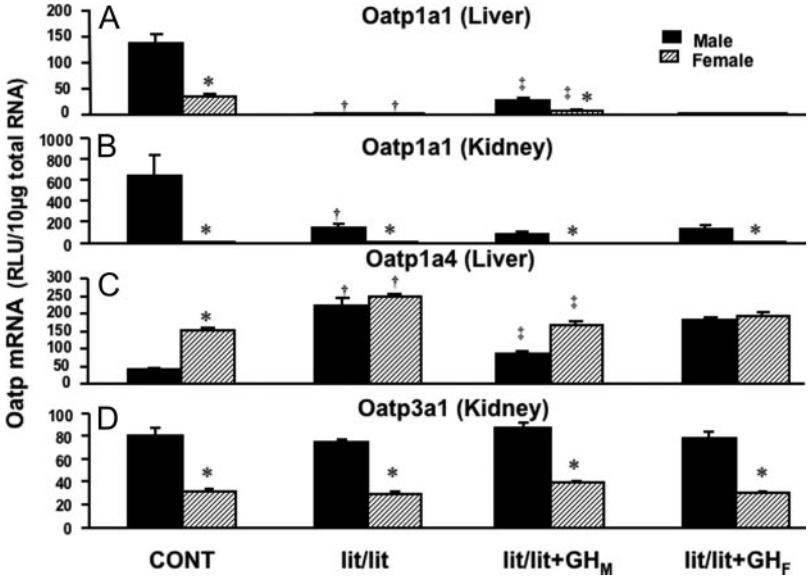

Fig. 3. Effects of GH on mouse Oatp1a1, Oatp1a4, and Oatp3a1 mRNA expression in liver and kidney tissues from control and *lit/lit* male and female mice. Total liver or kidney RNA was isolated and analyzed by the bDNA signal amplification assay for each Oatp mRNA content. The data are presented as mean RLU ± S.E.M. ($n = 5$). *Lit/lit,* placebo administered to *lit/lit* mice; *Lit/lit* + GH_M, rat GH twice daily administered by i.p. injection to *lit/lit* mice mimicking male-pattern GH secretion; *Lit/lit* + GH_F, 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.

Oatp1a1 transcripts decreased in males and remained at low levels in females (Fig. 3B). GH replacement in either male or female pattern did not alter Oatp1a1 mRNA expression in *lit/lit* mouse kidneys.

Hepatic Oatp1a4 mRNA expression in mice is female-predominant (Fig. 3C). Similar to HX mice, hepatic Oatp1a4 mRNA levels were much higher in male *lit/lit* mice than in wild-type mice, and the gender difference was abolished in *lit/lit* mice. Male-pattern GH replacement in *lit/lit* mice decreased hepatic Oatp1a4 mRNA expression in both males and females; however, female-pattern GH replacement had no effect.

Oatp3a1 mRNA abundance is 2.5-fold higher in male mouse kidneys than female kidneys (Fig. 3D). In *lit/lit* mice, renal Oatp3a1 mRNA expression remained at similar levels as in control mice and was not altered by either male- or female-pattern GH administration.

Discussion

Hormonal regulation of biotransformation enzymes and transporters is a critical aspect of both basal and inducible gene expression in liver and kidney. For example, during pregnancy, several endocrine hormones, such as estrogens, prolactin, and GH, are markedly altered (Soares, 2004). Hormonal alterations in hepatic or renal transporter expression can sometimes lead to pathological conditions, such as intrahepatic cholestasis (Vore et al., 1997). Therefore, knowledge of the contributions of individual hormones to Oatp expression will enable us to better understand how these transporters are regulated under normal and pathological hormone levels. Thus, in the present study, the mechanism of hormonal regulation of gender-divergent expression of certain Oatp family members was examined.

Constitutive expression of some Oatp transporters can be altered by sex hormones and/or GH. Castration decreases Oatp1a1 in liver and kidney and Oatp3a1 in kidney, but increases hepatic Oatp1a4 mRNA expression in male mice (Fig. 1). This indicates that androgens can alter basal Oatp expression in mice by stimulating Oatp1a1 and Oatp3a1, yet inhibiting hepatic Oatp1a4. Conversely, only minor contributions by estrogens to basal Oatp expression in mice were observed. Lack of circulating GH in *lit/lit* mice decreased Oatp1a1 but increased hepatic Oatp1a4 mRNA expression (Fig. 3). In the absence of GH, renal Oatp1a1 mRNA expression decreased in male but not in female mice. Both male- and female-pattern GH replacement could not restore renal Oatp1a1 expression (Fig. 3). In contrast, GH had no effect on renal Oatp3a1 mRNA expression (Fig. 3). Thus, GH secretion patterns regulate hepatic but not renal Oatp expression.

Oatp1a1 (previously named Oatp1) is an uptake transporter primarily expressed in rodent liver and kidney. In rats, no gender differences in hepatic Oatp1a1 protein expression exist (Rost et al., 2005), whereas in rat kidney, Oatp1a1 protein expression is higher in males than females (Gotoh et al., 2002). In mouse liver and kidney, Oatp1a1 mRNA expression is higher in males than in females (Cheng et al., 2005a), and renal Oatp1a1 expression has been shown to be androgen-dependent (Lu et al., 1996; Melia et al., 1998; Isern et al., 2001). The present study shows that liver and kidney Oatp1a1 is male-predominant because of stimulatory effects of androgens (Fig. 1). In addition, male-pattern GH secretion increases Oatp1a1 expression in mouse liver but not in kidney (Figs. 2 and 3).

Oatp1a4 (previously named Oatp2) is also an uptake transporter that is primarily expressed in liver and brain of rodents (Li et al., 2002; Cheng et al., 2005a). In rats, no gender differences in hepatic Oatp1a4 mRNA expression exist (Li et al., 2002), but conflicting data have been reported regarding hepatic Oatp1a4 protein expression. Guo et al. (2002a) showed hepatic Oatp1a4 protein expression is higher in females than males, whereas Rost et al. (2005) reported that hepatic Oatp1a4 protein expression is higher in males than females. In mice, Oatp1a4 is higher in female than male mouse livers (Cheng et al., 2005a). The present study showed that female-predominant Oatp1a4 mRNA expression in mouse liver is caused by inhibitory effects of male-pattern GH secretion and androgens (Table 1).

Oatp3a1 (previously named Oatp11) is highly expressed in kidney of both rats and mice (Melia et al., 1998; Cheng et al., 2005a). In rats, renal Oatp3a1 mRNA expression is male-predominant because of the stimulatory effects of androgens (Melia et al., 1998). In mice, male-predominant kidney Oatp3a1 mRNA expression is also caused by the stimulatory effect of androgens (Table 1).

In the present study, male-pattern GH replacement increases Oatp1a1 but decreases Oatp1a4 mRNA levels in livers of both HX mice and *lit/lit* mice (Table 1). However, androgens can only increase hepatic Oatp1a and decrease Oatp1a4 in pituitary-intact gonadectomized mice, but not in HX mice, as summarized in Table 1. These data indicate that in mouse liver, male-pattern GH secretion plays the primary role in hormonal regulation of Oatp1a1 and Oatp1a4. However, it has also been shown that sex hormones are capable of regulating gender-specific GH secretion patterns by influencing: 1) GH-releasing factor levels, 2) somatostatin synthesis and secretion, and 3) pituitary function (Legraverend et al., 1992; Painson et al., 1992). Thus, sex hormones can influence hepatic gene expression by modifying GH secretion patterns. Therefore, gender-divergent expression of Oatp1a1 and Oatp1a4 in mouse liver is caused by combined effects of both male-pattern GH secretion and androgens (Fig. 4).

Signaling mechanisms other than classic hormone receptor pathways may also be involved in regulation of hepatic male-predominant Oatp1a1 and female-predominant Oatp1a4. For example, although estrogens bind primarily to the estrogen receptor, they can also activate constitutive androstane receptor (CAR). Estrogens have been shown to activate mouse CAR by increasing nuclear translocation of CAR and

TABLE 1
Summary of gender-divergent regulation of Oatps in mouse liver and kidney

	GNX	Lit/lit	HX	
	Androgens	MP-GH	Androgens	MP-GH
Oatp1a1 (liver)	↑	↑	0	↑
Oatp1a1 (kidney)	↑	0	↑	0
Oatp1a4 (liver)	↓	↓	0	↓
Oatp3a1 (kidney)	↑	0	↑	0

GNX indicates study done in gonadectomized mice; Lit/lit, study done in *lit/lit* mice; HX, study done in hypophysectomized mice; Androgens, 5α-dihydrotestosterone was administered to gonadectomized or hypophysectomized mice; MP-GH, male-pattern GH administration to *lit/lit* or hypophysectomized mice; ↑, up-regulation in gene mRNA expression compared with placebo administration; 0, no alteration in gene mRNA expression compared with placebo administration; ↓, down-regulation in gene mRNA expression compared with placebo administration.

thus induce hepatic Cyp2b10 (Kawamoto et al., 2000). Previous studies showed that CAR activators (TCPOBOP, phenobarbital, and diallyl sulfate) markedly decrease mouse hepatic Oatp1a1 mRNA levels (Cheng et al., 2005b). In general, CAR agonists regulate target genes via affecting CAR nuclear translocation. Therefore, estrogens may decrease hepatic Oatp1a1 via CAR activation in female mice mimicking TCPOBOP and phenobarbital, contributing to male predominance. However, estrogens differently regulate Oatp1a4 expression. Estrogens suppress Oatp1a4 mRNA expression in ovariectomized female mice (Fig. 1C) but increase Oatp1a4 mRNA expression in HX male and female mice (Fig. 2C). Pregnane X receptor (PXR) activation represents a classic pathway for hepatic Oatp1a4 induction (Guo et al., 2002b; Cheng et al., 2005b). Cholestatic levels of estrogens decrease hepatic Oatp1a4 expression in rats by diminishing PXR binding to regulatory regions of Oatp1a4 (Geier et al., 2002). Therefore, in ovariectomized female mice, estrogens suppress Oatp1a4 mRNA expression, probably also mediated by diminishing PXR binding to regulatory regions of Oatp1a4. However, further experiments are required to explain why hepatic Oatp1a4 expression is suppressed in ovariectomized mice but induced in HX female mice.

Gender differences in pharmacokinetics and pharmacodynamics of chemicals are well documented in laboratory animals (Morris et al., 2003; Simon et al., 2004) and humans (Morris et al., 2003). It is not known whether gender-dimorphic expression of OATP exists in humans. However, mouse Oatp3a1 has the human ortholog OATP3A1 (Hagenbuch and Meier, 2003).

In summary, androgens and male-pattern GH secretion play important roles in the regulation of gender-dimorphic expression of mouse Oatp mRNA, as summarized in Table 1. In livers, male-predominant Oatp1a1 expression and female-predominant Oatp1a4 expression are caused by the stimulatory or inhibitory effects of male-pattern GH secretion and androgens, respectively. In kidneys, male-predominant Oatp1a1 and Oatp3a1 expression is solely androgen-dependent. In conclusion, gender-divergent Oatp expression in mice is primarily mediated by androgens and male-pattern GH secretion, with little contribution by female-pattern GH or estrogen.

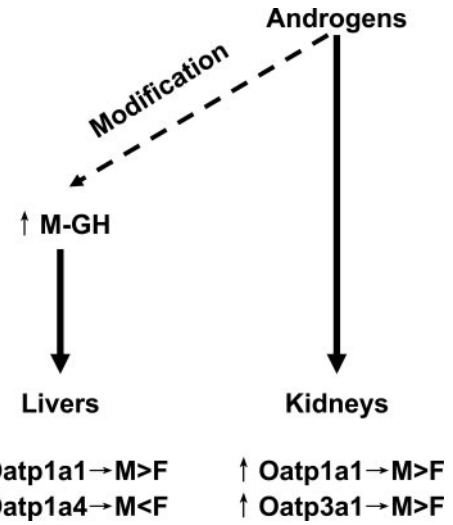

Fig. 4. Proposed mechanism of regulation of mouse Oatp1a1, Oatp1a4, and Oatp3a1 mRNA expression in mouse liver and kidney by androgens and male-pattern GH secretion. In mouse kidneys, androgens but not GH directly increased Oatp1a1 and Oatp3a1 mRNA levels. However, in mouse livers, male-pattern GH secretion plays the primary role in hormonal regulation of Oatp1a1 and Oatp1a4. Androgens may increase Oatp1a1 and decrease Oatp1a4 by maintaining male-pattern GH secretion because androgens are unable to alter these genes in the absence of intact pituitary function.

References

Aida K and Negishi M (1993) A trans-acting locus regulates transcriptional repression of the female-specific steroid 15 alpha-hydroxylase gene in male mice. *J Mol Endocrinol* **11:**213–222.

Beamer WH and Eicher EM (1976) Stimulation of growth in the little mouse. *J Endocrinol* **71:**37–45.

Beato M (1989) Gene regulation by steroid hormones. *Cell* **56:**335–344.

Bergwerk AJ, Shi X, Ford AC, Kanai N, Jacquemin E, Burk RD, Bai S, Novikoff PM, Stieger B, Meier PJ, et al. (1996) Immunologic distribution of an organic anion transport protein in rat liver and kidney. *Am J Physiol* **271:**G231–G238.

Buist SC, Cherrington NJ, Choudhuri S, Hartley DP, and Klaassen CD (2002) Gender-specific and developmental influences on the expression of rat organic anion transporters. *J Pharmacol Exp Ther* **301:**145–151.

Buist SC and Klaassen CD (2004) Rat and mouse differences in gender-predominant expression of organic anion transporter (Oat1–3; Slc22a6–8) mRNA levels. *Drug Metab Dispos* **32:**620–625.

Chen C and Klaassen CD (2004) Rat multidrug resistance protein 4 (Mrp4, Abcc4): molecular cloning, organ distribution, postnatal renal expression, and chemical inducibility. *Biochem Biophys Res Commun* **317:**46–53.

Cheng TC, Beamer WG, Phillips JA 3rd, Bartke A, Mallonee RL, and Dowling C (1983) Etiology of growth hormone deficiency in little, Ames, and Snell dwarf mice. *Endocrinology* **113:**1669–1678.

Cheng X, Maher J, Chen C, and Klaassen CD (2005a) Tissue distribution and ontogeny of mouse organic anion transporting polypeptides (Oatps). *Drug Metab Dispos* **33:**1062–1073.

Cheng X, Maher JM, Dieter MZ, and Klaassen CD (2005b) Regulation of mouse organic anion-transporting polypeptides (oatps) in liver by prototypical microsomal enzyme inducers that activate distinct transcription factor pathways. *Drug Metab Dispos* **33:**1276–1282.

Gartung C and Matern S (1997) Molecular regulation of sinusoidal liver bile acid transporters during cholestasis. *Yale J Biol Med* **70:**355–363.

Geier A, Kim SK, Gerloff T, Dietrich CG, Lammert F, Karpen SJ, Stieger B, Meier PJ, Matern S, and Gartung C (2002) Hepatobiliary organic anion transporters are differentially regulated in acute toxic liver injury induced by carbon tetrachloride. *J Hepatol* **37:**198–205.

Gotoh Y, Kato Y, Stieger B, Meier PJ, and Sugiyama Y (2002) Gender difference in the Oatp1-mediated tubular reabsorption of estradiol 17beta-D-glucuronide in rats. *Am J Physiol* **282:**E1245–E1254.

Guo GL, Johnson DR, and Klaassen CD (2002a) Postnatal expression and induction by pregnenolone-16α-carbonitrile of the organic anion-transporting polypeptide 2 in rat liver. *Drug Metab Dispos* **30:**283–288.

Guo GL, Staudinger J, Ogura K, and Klaassen CD (2002b) Induction of rat organic anion transporting polypeptide 2 by pregnenolone-16α-carbonitrile is via interaction with pregnane X receptor. *Mol Pharmacol* **61:**832–839.

Hagenbuch B and Meier PJ (2003) The superfamily of organic anion transporting polypeptides. *Biochim Biophys Acta* **1609:**1–18.

Hagenbuch B and Meier PJ (2004) Organic anion transporting polypeptides of the OATP/ SLC21 family: phylogenetic classification as OATP/ SLCO superfamily, new nomenclature and molecular/functional properties. *Pflueg Arch Eur J Physiol* **447:**653–665.

Hartley DP and Klaassen CD (2000) Detection of chemical-induced differential expression of rat hepatic cytochrome P450 mRNA transcripts using branched DNA signal amplification technology. *Drug Metab Dispos* **28:**608–616.

Isern J, Hagenbuch B, Stieger B, Meier PJ, and Meseguer A (2001) Functional analysis and androgen-regulated expression of mouse organic anion transporting polypeptide 1 (Oatp1) in the kidney. *Biochim Biophys Acta* **1518:**73–78.

Jansson JO, Downs TR, Beamer WG, and Frohman LA (1986) Receptor-associated resistance to growth hormone-releasing factor in dwarf "little" mice. *Science (Wash DC)* **232:**511–512.

Kasukawa Y, Baylink DJ, Guo R, and Mohan S (2003) Evidence that sensitivity to growth hormone (GH) is growth period and tissue type dependent: studies in GH-deficient lit/lit mice. *Endocrinology* **144:**3950–3957.

Kato Y, Kuge K, Kusuhara H, Meier PJ, and Sugiyama Y (2002) Gender difference in the urinary excretion of organic anions in rats. *J Pharmacol Exp Ther* **302:**483–489.

Kawamoto T, Kakizaki S, Yoshinari K, and Negishi M (2000) Estrogen activation of the nuclear orphan receptor CAR (constitutive active receptor) in induction of the mouse Cyp2b10 gene. *Mol Endocrinol* **14:**1897–1905.

Kimura N, Arai K, Sahara Y, and Suzuki H (1994) Estradiol transcriptionally and posttranscriptionally up-regulates thyrotropin-releasing hormone receptor messenger ribonucleic acid in rat pituitary cells. *Endocrinology* **134:**432–440.

Kudo N, Katakura M, Sato Y, and Kawashima Y (2002) Sex hormone-regulated renal transport of perfluorooctanoic acid. *Chem Biol Interact* **139:**301–316.

Laatikainen TJ (1975) Fetal bile acid levels in pregnancies complicated by maternal intrahepatic cholestasis. *Am J Obstet Gynecol* **122:**852–856.

Legraverend C, Mode A, Wells T, Robinson I, and Gustafsson JA (1992) Hepatic steroid hydroxylating enzymes are controlled by the sexually dimorphic pattern of growth hormone secretion in normal and dwarf rats. *FASEB J* **6:**711–718.

Li N, Hartley DP, Cherrington NJ, and Klaassen CD (2002) Tissue expression,

ontogeny, and inducibility of rat organic anion transporting polypeptide 4. *J Pharmacol Exp Ther* **301:**551–560.
Lin SC, Lin CR, Gukovsky I, Lusis AJ, Sawchenko PE, and Rosenfeld MG (1993) Molecular basis of the little mouse phenotype and implications for cell type-specific growth. *Nature (Lond)* **364:**208–213.
Lu R, Kanai N, Bao Y, Wolkoff AW, and Schuster VL (1996) Regulation of renal oatp mRNA expression by testosterone. *Am J Physiol* **270:**F332–F337.
MacLeod JN, Pampori NA, and Shapiro BH (1991) Sex differences in the ultradian pattern of plasma growth hormone concentrations in mice. *J Endocrinol* **131:**395–399.
Melia MJ, Bofill N, Hubank M, and Meseguer A (1998) Identification of androgen-regulated genes in mouse kidney by representational difference analysis and random arbitrarily primed polymerase chain reaction. *Endocrinology* **139:**688–695.
Morris ME, Lee HJ, and Predko LM (2003) Gender differences in the membrane transport of endogenous and exogenous compounds. *Pharmacol Rev* **55:**229–240.
Nakamura S, Sugawara Y, Fukushima T, Ito Y, Ohashi M, and Takaiti O (1993) Disposition and metabolic fate of clentiazem in rats and dogs. *Biol Pharm Bull* **16:**647–655.
Noshiro M and Negishi M (1986) Pretranslational regulation of sex-dependent testosterone hydroxylases by growth hormone in mouse liver. *J Biol Chem* **261:**15923–15927.
Painson JC, Thorner MO, Krieg RJ, and Tannenbaum GS (1992) Short-term adult exposure to estradiol feminizes the male pattern of spontaneous and growth hormone-releasing factor-stimulated growth hormone secretion in the rat. *Endocrinology* **130:**511–519.
Paul SJ, Ortolano GA, Haisenleder DJ, Stewart JM, Shupnik MA, and Marshall JC (1990) Gonadotropin subunit messenger RNA concentrations after blockade of gonadotropin-releasing hormone action: testosterone selectively increases follicle-stimulating hormone beta-subunit messenger RNA by posttranscriptional mechanisms. *Mol Endocrinol* **4:**1943–1955.
Pauli-Magnus C and Meier PJ (2005) Hepatocellular transporters and cholestasis. *J Clin Gastroenterol* **39 (Suppl 2):**S103–S110.
Rost D, Kopplow K, Gehrke S, Mueller S, Friess H, Ittrich C, Mayer D, and Stiehl A (2005) Gender-specific expression of liver organic anion transporters in rat. *Eur J Clin Investig* **35:**635–643.
Sato M, Suzaka H, and Miyazaki H (2000) Sex-related differences in urinary excretion of egualen sodium in rats. *Drug Metab Dispos* **28:**21–27.
Saunders A, Terry LC, Audet J, Brazeau P, and Martin JB (1976) Dynamic studies of growth hormone and prolactin secretion in the female rat. *Neuroendocrinology* **21:**193–203.
Simon FR, Fortune J, Iwahashi M, Qadri I, and Sutherland E (2004) Multihormonal regulation of hepatic sinusoidal Ntcp gene expression. *Am J Physiol* **287:**G782–G794.
Soares MJ (2004) The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal-fetal interface. *Reprod Biol Endocrinol* **2:**51.
Tanaka Y, Kadoh Y, Mukumoto S, and Ishikawa H (1991) The role of age and sex hormones on the urinary excretion of zenarestat in rats. *Xenobiotica* **21:**1273–1279.
Tanaka Y, Slitt AL, Leazer TM, Maher JM, and Klaassen CD (2005) Tissue distribution and hormonal regulation of the breast cancer resistance protein (Bcrp/Abcg2) in rats and mice. *Biochem Biophys Res Commun* **326:**181–187.
Tannenbaum GS and Martin JB (1976) Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. *Endocrinology* **98:**562–570.
Terashita S, Sawamoto T, Tozuka Z, Tokuma Y, and Hata T (1994) Interaction of renal excretion between nilvadipine metabolites, M3 and M7 in rats: characterization of sex-dependent and sex-independent active secretion in the kidney. *Res Commun Mol Pathol Pharmacol* **86:**205–215.
Urakami Y, Nakamura N, Takahashi K, Okuda M, Saito H, Hashimoto Y, and Inui K (1999) Gender differences in expression of organic cation transporter OCT2 in rat kidney. *FEBS Lett* **461:**339–342.
Vore M, Liu Y, and Huang L (1997) Cholestatic properties and hepatic transport of steroid glucuronides. *Drug Metab Rev* **29:**183–203.
Waxman DJ, Pampori NA, Ram PA, Agrawal AK, and Shapiro BH (1991) Interpulse interval in circulating growth hormone patterns regulates sexually dimorphic expression of hepatic cytochrome P450. *Proc Natl Acad Sci USA* **88:**6868–6872.
Wood M, Ananthanarayanan M, Jones B, Wooton-Kee R, Hoffman T, Suchy FJ, and Vore M (2005) Hormonal regulation of hepatic organic anion transporting polypeptides. *Mol Pharmacol* **68:**218–225.

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