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Tissue Distribution, Ontogeny, and Hormonal Regulation of Xenobiotic Transporters in Mouse Kidneys

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ABSTRACT:

Kidneys play important roles in the elimination of numerous endogenous and exogenous chemicals. In recent years, at least 37 xenobiotic transporters have been identified in mammalian kidneys. Although much progress has been made, information on 14 of these transporters (ATP-binding cassette [Abc] a1, apical sodium bile acid transporter [Asbt], breast cancer resistance protein, concentrative nucleoside transporter 1, equilibrative nucleoside transporter [Ent] 2, Ent3, sodium-phosphate cotransporter [Npt] 1, Npt2a, Npt2b, Npt2c, organic anion transporter [Oat] 5, organic anion-transporting polypeptide [Oatp] 4c1, peptide transporter 2, and uric acid transporter [Urat] 1) in kidneys is quite limited. Therefore, the purpose of the present study was to examine the tissue distribution, ontogeny, and hormonal regulation of these 14 transporters in kidneys of mice. Other than in kidneys, Npt2b is also highly expressed in liver and lung, Npt2c in liver and colon, Asbt in ileum, and Abca1 in liver, lung, testis, ovary, and placenta of mice. Most of these (13 of 14) transporters are lowly expressed in mouse kidneys until 15 days of age, which in part contributes to the immaturity of excretory function in fetal and newborn kidneys. One exception is Ent2, which is highly expressed before birth and gradually decreases after birth until reaching adult levels at 15 days of age. Gender-divergent expression of male-predominant (Urat1 and Oatp4c1) and female-predominant (Oat5) transporters in mouse kidneys is primarily due to stimulatory effects of androgens and estrogens, respectively. In conclusion, the mRNA expression of xenobiotic transporters in kidneys is determined by tissue, age, and sex hormones.

Kidneys are pivotal in the elimination of numerous xenobiotics, including drugs and environmental chemicals, as well as endogenous metabolites. Kidneys have developed transport systems to prevent urinary loss of filtered nutrients, such as glucose, oligopeptides, and inorganic ions, as well as to facilitate the elimination of a variety of xenobiotics (Fig. 1).

The excretory transport processes responsible for the renal tubular secretion of xenobiotics are performed by two distinctively localized transporters: basolateral excretion transporters and apical excretion transporters (Fig. 1). The basolateral transporters responsible for the renal tubular uptake of substrates from blood in mice include the organic anion transporter (Oat) 1 and 3, organic cation transporter (Oct) 1 and 2, and the organic anion-transporting polypeptide (Oatp) 4c1. The apical efflux transporters are ATP-dependent active transporters and include P-glycoproteins (or multidrug resistance protein 1b), multidrug resistance-associated proteins (Mrp) 2 and 4, breast cancer resistance protein (Bcrp), and multidrug and toxin extrusion 1. During the last decade, considerable progress has been made regarding the identification and characterization of these organic anion and cation transporters.

In addition to the transporters for the efflux of chemicals from blood to filtrate, there are also reuptake transporters at both the apical and basolateral membranes of the proximal tubule cells. For example, apical sodium bile-acid transporter (Asbt), nucleotide transporter (Cnt1), organic anion and cation transporters [sodium-phosphate cotransporter (Npt) 1, 2a, and 2c, Oat2 and 5, Oatp1a1, 1a4, 1a6, 2a1, 2b1, and 3a1, Octn1 and n2, and uric acid transporter (Urat) 1], and peptide transporter (Pept2) are localized in the apical membrane of proximal tubular cells responsible for uptake of organic compounds from the glomerular filtrate into tubular cells. In the basolateral membrane of proximal tubular cells, retro-transporters including Abca1, equilibrative nucleoside transporter (Ent) 2 and 3, Mrp1, 3, 5, and 6, and organic solute transporter (Ost) α/β are responsible for transporting compounds from the kidney back into blood (Fig. 1).

Although the localization of many transporters in kidneys is known, limited information is available about the regulation of some of these transporters, which are noted in black in Fig. 1. Therefore, the purpose

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ABBREVIATIONS: Oat, organic anion transporter; Oct, organic cation transporter; Oatp, organic anion transporting polypeptide; Mrp, multidrug resistance-associated protein; Bcrp, breast cancer resistance protein; Asbt, apical sodium bile-acid transporter; Cnt, concentrative nucleoside transporter; Npt, sodium-phosphate cotransporter; Urat1, uric acid transporter 1; Pept, peptide transporter; Abca1, ATP-binding cassette (Abc) transporter a1; Ent, equilibrative nucleoside transporter; Ost, organic solute transporter; MOPS, 4-morpholinepropanesulfonic acid; DHT, 5α-dihydroxytestosterone; E2, estrogen; GH, growth hormone; *lit/lit*, mutation in growth hormone-releasing-hormone receptor gene; TEA, tetraethylammonium; bDNA, branched DNA signal amplification assay; WT, wild-type.

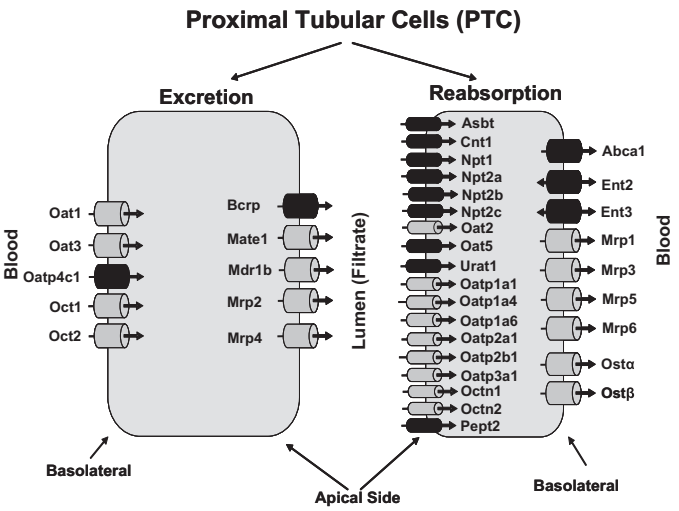

FIG. 1. Diagram of cellular localization and transport direction of known transporters in kidneys. Excretion transporters from basolateral and apical membranes are depicted in the proximal tubular cells (PTCs) of kidneys (left). Reabsorption transporters from basolateral and apical membranes are shown in the PTCs of kidneys (right). Transporters labeled in gray are transporters for which there are previously published data concerning tissue distribution, ontogeny, and gender difference. Transporters labeled in black are transporters that were not fully characterized previously and are addressed in the present study.

of this study was to determine the expression of these "less-studied" transporters in mouse kidneys, with regard to tissue distribution, ontogeny, and gender-divergent expression 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 Diagnostics (Indianapolis, IN). Formaldehyde, MOPS, sodium citrate, and sodium bicarbonate were purchased from Fisher Chemicals (Fairlawn, NJ). Chloroform, agarose, and ethidium bromide were purchased from AMRESCO Inc. (Solon, OH). Rat growth hormone was obtained through Dr. Albert F. Parlow at the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases, Harbor-UCLA Medical Center (Torrance, CA). Pellets for subcutaneous release of the hormones used in this study, 5α-dihydroxytestosterone (DHT), 17β-estradiol (E2), growth hormone (GH), and placebo were purchased from Innovative Research of America (Sarasota, FL). All other chemicals, unless otherwise indicated, were purchased from Sigma-Aldrich.

Animals and Breeding. Eight-week-old adult male and female C57BL/6 mice (n = 6/gender) were purchased from The Jackson Laboratory (Bar Harbor, Maine) and housed according to American Animal Association Laboratory Animal Care guidance. For tissue distribution, 12 tissues (liver, kidney, lung, stomach, duodenum, jejunum, ileum, colon, heart, brain, testis, and ovary) were collected. Placenta was removed from pregnant mice on day −2. The small intestine was longitudinally dissected, rinsed in saline, and divided into three equal-length sections (duodenum, jejunum, and ileum), before being snap-frozen in liquid nitrogen. For the ontogeny study, mice were bred in the animal facilities at the University of Kansas Medical Center. Kidneys from male and female C57BL/6 mice were collected at −2, 0, 5, 10, 15, 22, 30, 35, 40, and 45 days of age (n = 5/gender/age), snap-frozen in liquid nitrogen, and stored at −80°C.

Sex Hormone Administration to Gonadectomized Mice. C57BL/6 mice were castrated or ovariectomized at 37 days of age by Charles River Laboratories (Wilmington, MA). 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 subcutaneously in the gonadectomized male and female mice. The mice were separated into four groups (n = 6–7/gender/treatment): 1) gonadectomized mice (castration in males and ovariectomy in females) + placebo, 2) gonadectomized mice + DHT, 3) gonadectomized mice + E2, and 4) placebo-treated, age-matched mice, which were used as controls. Kidneys were collected at 64 days of age from gonadectomized and age-matched control mice.

Growth Hormone Administration to *lit/lit* Mice. Growth hormone-releasing hormone receptor mutant heterozygous mice (C57BL/6J-Ghrhr^{lit}) were purchased from The Jackson Laboratory. The mice (n = 6/group) were treated for 10 days with rat GH in a male pattern (twice daily, intraperitoneal injection, dose of 2.5 mg of GH/kg/day), a female pattern (continuous infusion via subcutaneously implanted 21-day-release 1-mg rat GH pellet), and placebo. After treatment, kidneys were collected for total RNA isolation.

Total RNA Isolation. Total RNA was isolated using RNA-Bee reagents (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.

Development of Specific Oligonucleotide Probe Sets for Branched DNA Analysis. Gene sequences of interest were accessed from GenBank. The strategy of multiple oligonucleotide probe set design has been described previously (Hartley and Klaassen, 2000). Oligonucleotide probe sets of mouse Npt1, 2a, 2b, 2c, Oat5, and Urat1 (including capture extenders, label extenders, and blockers) are shown in Table 1. Probe sets of mouse Abca1, Asbt (Cheng and Klaassen, 2006), Bcrp (Tanaka et al., 2005), Cnt1, Ent2, Ent4 (Lu et al., 2004), Pept2 (Lu and Klaassen, 2006), and Oatp4c1 (Cheng et al., 2005) have been reported previously. Probe sets were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA).

bDNA Assay. Reagents required for RNA analysis (i.e., lysis buffer, amplifier/label probe dilution buffer, and substrate solution) were supplied in the Quantigene bDNA signal amplification kit (Panomics Inc., Fremont, CA). Each transporter mRNA expression was analyzed according to the method reported previously (Hartley and Klaassen, 2000). Data are presented as relative light units per 10 μg of total RNA.

Statistical Analysis. Data are expressed as mean ± S.E.M. Data obtained from the effects of sex hormones and growth hormones on transporter expression in mouse kidneys were analyzed by one-way analysis of variance, followed by Duncan's post hoc test. Data for gender differences in the tissue distribution study and ontogeny study were analyzed by Student's t test. Statistical significance was considered at $p < 0.05$.

Results

Tissue Distribution of Xenobiotic Transporters. Of the 14 less-studied renal transporters, the tissue distributions of six of these transporters have been described previously, specifically, Oatp4c1 (Cheng et al., 2005), Bcrp (Tanaka et al., 2005), Cnt1, Ent2, Ent3 (Lu et al., 2004), and Pept2 (Lu and Klaassen, 2006). Thus, the mRNA expression of the remaining eight transporters was quantified in 13 mouse tissues (Figs. 2 and 3). Npts are important in maintaining phosphate homeostasis in vertebrates and in transport of β-lactam antibiotics as well as inorganic anions (Yabuuchi et al., 1998). Npt1 mRNA expression was highest in mouse kidney but was much lower in liver and other tissues. A gender difference in Npt1 mRNA expression was observed in mouse kidneys, with 33% higher levels in males. Npt2a mRNA (Fig. 2) was almost exclusively expressed in mouse kidneys, with 43% higher levels in kidneys of male than female mice. Npt2b mRNA expression (Fig. 2) was low in all tissues examined, with the highest levels in liver, modest levels in lung, heart, and brain, and lower levels in other tissues. Npt2c mRNA expression was highest in mouse kidneys, followed by colon and liver, and low in other tissues. There are gender differences in Npt2c mRNA expression in mouse liver (female predominance) and heart (male predominance) (Fig. 2).

Abca1, also named cholesterol efflux regulatory protein, was expressed in many tissues, with the highest levels in mouse placenta and ovary, followed by lung, liver, and testis, and lower levels in other tissues (Fig. 3). Abca1 mRNA expression in livers was higher in male

TABLE 1

Oligonucleotide probe sets generated for analysis of mouse transporter mRNA expression by the branched DNA signal amplification assay

Function[a]	Probe Sequence
Npt1 (NM_009198)[b]	
CE	ctgcctcgttctaagggaggtTTTTTctcttggaaagaaagt
CE	tggcctgttgcatgagggaTTTTTctcttggaaagaaagt
CE	ggataatggcccagagtggaaTTTTTctcttggaaagaaagt
LE	tgctggcctgttgacactgttTTTTTaggcataggacccgtgtct
LE	gcccatttgacccatatttcaTTTTTaggcataggacccgtgtct
LE	aaccctgatagagtcatagaggtaagtTTTTTaggcataggacccgtgtct
LE	ggacaataaatggtcccattacaTTTTTaggcataggacccgtgtct
LE	tgctcatatacgggtggtccttTTTTTaggcataggacccgtgtct
LE	ggatatgatgtagtccttctcactgcTTTTTaggcataggacccgtgtct
LE	cagggattgtctgcctgagcTTTTTaggcataggacccgtgtct
LE	gagatttaagcatagctttgattggTTTTTaggcataggacccgtgtct
LE	tgtatgtaaccaggagactgttcgTTTTTaggcataggacccgtgtct
LE	gggaggctggacagcagtcTTTTTaggcataggacccgtgtct
LE	ccacagatgtaggcaagcagatagTTTTTaggcataggacccgtgtct
LE	gtctgacatctgacctgctaggataTTTTTaggcataggacccgtgtct
BL	cagatgaagccactcacaagca
BL	gggccagcccagaagatca
BL	aacaataccaaagatgtagaagaccat
BL	caggaaagactcagaacacaccc
BL	ggggtcatcaaagaatagaaagaac
BL	accatatgaaagcaaagctattga
BL	cagtgctgataaatgtcggcg
BL	cattctctctaacattaacatgaagca
Npt2a (NM_011392)	
CE	ccccaatctctcgctgtaggaTTTTTctcttggaaagaaagt
CE	acagctgtgctctgtgagggtTTTTTctcttggaaagaaagt
CE	gctcctcttcctgagccagctTTTTTctcttggaaagaaagt
LE	gttgccccatgcaccatgTTTTTaggcataggacccgtgtct
LE	ggctgggcacataagcaaagTTTTTaggcataggacccgtgtct
LE	atggcataggtggatgttccaTTTTTaggcataggacccgtgtct
LE	gaccgggctcagactggagTTTTTaggcataggacccgtgtct
LE	ccaggacttccccacagggTTTTTaggcataggacccgtgtct
LE	gcctgggctctggcttttTTTTTaggcataggacccgtgtct
LE	tgggcaagcttctgggacaTTTTTaggcataggacccgtgtct
LE	caggagcttcgtgccaaccTTTTTaggcataggacccgtgtct
LE	gccagcatcagaggcaccttTTTTTaggcataggacccgtgtct
BL	ggggagacagccggtcc
BL	tgtctcccacggactggaagt
BL	ggaatcctgtgcaggacttgtg
BL	tggtaggtagtgggtcatgacatt
Npt2b (NM_011402)	
CE	ggcagcggaactgcagcaTTTTTctcttggaaagaaagt
CE	cccagaagccttgaccggTTTTTctcttggaaagaaagt
LE	ggtacgcccacgcccacTTTTTaggcataggacccgtgtct
LE	ggagcagcagcaggatgatgTTTTTaggcataggacccgtgtct
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BL	cagcaacgcctctggaagc
BL	cagcacttgctgcagcgac
Npt2c (NM_080854)	
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CE	cagcggagagaaggtccaggTTTTTctcttggaaagaaagt
CE	tgccttgagaaagctgctgaTTTTTctcttggaaagaaagt
CE	tggaaagcagagctgaggatgtTTTTTctcttggaaagaaagt
LE	tccaccaggtcaaaggcatcTTTTTaggcataggacccgtgtct
LE	tgctggccatgccaacctTTTTTaggcataggacccgtgtct
LE	ggcttcccaggagtccacaTTTTTaggcataggacccgtgtct
LE	gccattttgctgcctagtagcTTTTTaggcataggacccgtgtct
LE	gacaaggactgtgaccagcacaTTTTTaggcataggacccgtgtct
LE	ccaccatgctgaccacgatagaTTTTTaggcataggacccgtgtct
LE	cctggacagtcagcaacttagaggTTTTTaggcataggacccgtgtct
BL	cctggaatggaaccggagatt
BL	gatctgtacctccctcttccaag
BL	ctttcagttggtcagcgttctt
BL	ccacccggcgaagcc

TABLE 1—*Continued.*

Function[a]	Probe Sequence
BL	ccagggagcagatgaaaaagtaga
BL	cattgtccttgaaaatgtctcca
BL	cacagggttggacagcacca
BL	ccaatgaccaagcccgc
BL	ggaagatgtgctggagctctg
Oat5 (NM_144785)	
CE	gggaagaatcccatagatgatccTTTTTctcttggaaagaaagt
CE	ttggtctcaggaagaagaagggTTTTTctcttggaaagaaagt
CE	ttttcctcttattttctatatcttggatTTTTTctcttggaaagaaagt
CE	ggccggttggaattctttataatTTTTTctcttggaaagaaagt
LE	caacgaggccaccaaggaaTTTTTaggcataggacccgtgtct
LE	ggtgtcactttggcaaccacaTTTTTaggcataggacccgtgtct
LE	gaccacgatatgctgaatgatatatatTTTTTaggcataggacccgtgtct
LE	ttatacatgacaaactagtataatttttaatttaaTTTTTaggcataggacccgtgtct
LE	gattttattcaaatttctttattatagttattTTTTTaggcataggacccgtgtct
BL	cgctcctccagcactgcca
BL	cgtgaggatcataagtagaggagacag
BL	agggcaaggaagcagagtatgt
BL	tgagtcaggcagaggctgattc
BL	tctttctttgcctcttttgagc
BL	tttttcatctttctccttgatcaga
BL	aaatcttgctaacatataggtataattctg
BL	ctcattatttatacagtgacttatatggaa
Urat1 (NM_009203)	
CE	tcgcctgggaggtgctgtTTTTTctcttggaaagaaagt
CE	ctctgtaagctgccattgaggtTTTTTctcttggaaagaaagt
CE	caggaagatggactgggccTTTTTctcttggaaagaaagt
LE	tgtccaggagaggcacccaTTTTTaggcataggacccgtgtct
LE	tccaaggtccccagggatacTTTTTaggcataggacccgtgtct
LE	ggaccaggtgggatggagaTTTTTaggcataggacccgtgtct
LE	ggcagcgtcactccagttggTTTTTaggcataggacccgtgtct
LE	ccatcctcgcatggctcagtTTTTTaggcataggacccgtgtct
LE	ggtgctatggtcgtaaacccagTTTTTaggcataggacccgtgtct
LE	gagttacataccaggtcccacgtTTTTTaggcataggacccgtgtct
LE	atgggtctcagggcctggTTTTTaggcataggacccgtgtct
LE	ggctcctaccaggattccagcTTTTTaggcataggacccgtgtct
LE	ggcatggccacacactgcTTTTTaggcataggacccgtgtct
LE	tgcgcccaaacctatctgaTTTTTaggcataggacccgtgtct
BL	cggccagaagaacatcggg
BL	tggtggggttgctggtca
BL	tgtcggaagcggaggcac
BL	tggccgtggcgttgga
BL	ggtcacgattgtggacctgaa

CE, capture extender; LE, label extender; BL, blocker.
[a] Function refers to the type of bDNA oligonucleotide probe represented by each sequence.
[b] GenBank accession numbers for each transcript are given in parenthesis after the gene name.

than in female mice but is higher in kidney and duodenum of female than male mice.

Asbt mRNA expression was predominant in mouse ileum, followed by kidney, with lower levels in other tissues. In kidneys and ilea of mice, Asbt mRNA expression was female-predominant (Fig. 3).

Oat5 and Urat1 are both Oat transporters and are almost exclusively expressed in kidneys of mice (Fig. 3). Oat5 mRNA expression was 100% higher in kidneys of female than male mice, whereas Urat1 was 70% higher in males than in females.

Ontogenic Expression of Transporters in Kidneys of Mice. Because Npt2b and Abca1 are lowly expressed in kidneys of mice (Figs. 1 and 2), the developmental expression of these two transporters in kidneys of mice was not characterized. As depicted in Fig. 1, the 12 other less-studied transporters are responsible for uptake of chemicals from blood into proximal tubule cells, efflux into filtrate, reabsorption from the filtrate, or efflux back into blood.

The ontogenic expression of uptake transporters from the filtrate into kidneys (uptake transporters) is shown in Figs. 4 and 5. Npt1, 2a, and 2c share a similar developmental pattern in kidneys, with relatively low levels until they reach adult expression by 15 days of age (Fig. 4). Asbt is expressed at very low levels in kidneys during the first 2 weeks of life, with higher levels in female mice by 6 weeks of age (Fig. 5). Cnt1 exhibits a gradual increase in expression during the first 45 days of life (Fig. 5). Pept2 is relatively low until it reaches mature expression by 15 days of age (Fig. 5). Oat5 has low fetal expression in kidneys, increases at birth, and remains relatively constant until day 22, when it increases more in female than in male mice, resulting in a gender difference in expression (Fig. 5). Urat1 (Enomoto et al., 2002) is expressed at low levels in kidneys during the first 10 days of age and then gradually increases over the first 6 weeks of life. At 22 days of age, Urat1 mRNA expression is higher in female than in male kidneys. Male-predominant expression of Urat1 is depicted in adult mice as reported previously (Hosoyamada et al., 2004) (Fig. 5).

Ent2 and 3 are two retro-transporters that efflux chemicals from kidneys back into blood (Lu et al., 2004). Ent2 is expressed at similar levels in mouse kidneys at various ages, but in general decreases as the animal matures, whereas Ent3 shows a continuous increase in expression from birth through day 45 (Fig. 6). Oatp4c1 transports xenobiotics, such as digoxin, from blood into proximal tubule cells (Mikkaichi et al., 2004). Oatp4c1 expression is relatively constant from before birth until 2 weeks of age, when it doubles to reach adult levels (Fig. 6). Oatp4c1 mRNA expression is higher in female than in male kidneys at 30 days of age. At 45 days of age, Oatp4c1 mRNA

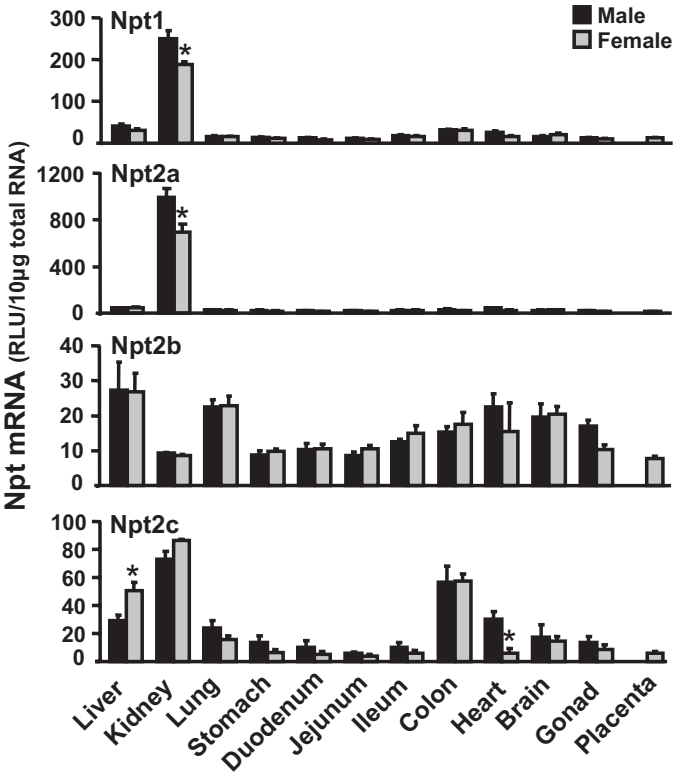

FIG. 2. Tissue distribution of Npt1, 2a, 2b, and 2c. Total RNA from male and female C57BL/6 mouse tissues ($n = 6$/gender) was analyzed by the bDNA assay for mRNA expression of each Npt. Data are presented as mean ± S.E.M. *, statistically significant difference between male and female mice ($p < 0.05$). RLU, relative light units.

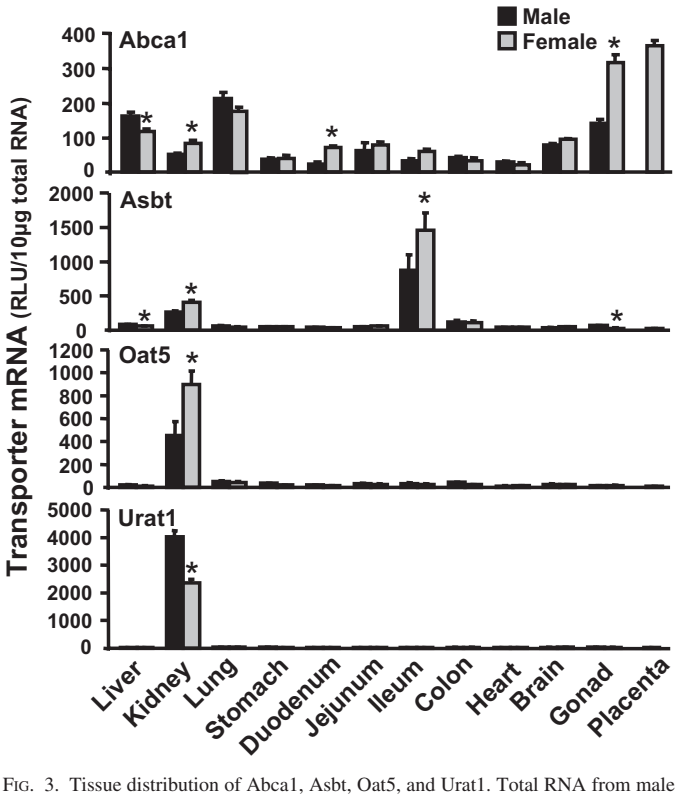

FIG. 3. Tissue distribution of Abca1, Asbt, Oat5, and Urat1. Total RNA from male and female C57BL/6 mouse tissues ($n = 6$/gender) was analyzed by the bDNA assay for mRNA expression of each transporter. Data are presented as mean ± S.E.M. *, statistically significant difference between male and female mice ($p < 0.05$). RLU, relative light units.

expression is male-predominant in mouse kidneys. Bcrp, a proximal tubule efflux transporter into the filtrate, is lowly expressed in kidneys before birth, gradually increases after birth, and reaches adult levels at 22 days of age (Fig. 6).

Effects of Sex Hormones on Regulation of Gender-Divergent Expression of Oat5, Urat1, and Oatp4c1 in Kidneys of Mice. Of the 14 less-studied renal transporters, tissue distribution and ontogeny studies showed that Npt1, Npt2a, Urat1, and Oatp4c1 are male-predominant (Cheng et al., 2005), whereas Abca1, Asbt, Oat5, and Pept2 are female-predominant in kidneys of adult mice (Figs. 2–6).

Regulatory mechanisms for the gender-dimorphic expression of Oat5, Urat1, and Oatp4c1 transporters by sex hormones were further determined in kidneys of mice (Fig. 7). Oat5 mRNA expression is female-predominant in kidneys of mice (Fig. 3). Gonadectomy (removal of testes in males or removal of ovaries in females) attenuates the gender-different expression of Oat5. In gonadectomized mice, estrogen replacement increased Oat5 mRNA levels, whereas androgen replacement did not alter Oat5 mRNA expression. Therefore, female-predominant Oat5 expression in kidneys of mice is due to the stimulatory effect of estrogens.

Urat1 mRNA expression is male-predominant in mouse kidneys (Figs. 3 and 5). Gonadectomy abolished gender-different expression of Urat1. In gonadectomized mice, androgen administration doubled Urat1 mRNA in both male and female mice (Fig. 7), whereas estrogens produced a small decrease in Urat1 mRNA (Fig. 7). Therefore, male-predominant Urat1 expression in kidneys of mice is due to the stimulatory effects of androgens.

Kidney Oatp4c1 mRNA expression is male-predominant in adult mice (Fig. 6), consistent with a previous study (Cheng et al., 2005). Gonadectomy converted male-predominant Oatp4c1 mRNA expression to female-predominance in mouse kidneys (Fig. 7). In gonadectomized mice, estrogen administration had no effect, whereas androgen administration increased Oatp4c1 mRNA levels in both male and female mice (Fig. 7). Thus, male-predominant Oatp4c1 expression in kidneys of mice is primarily due to stimulatory effect of androgens.

Effects of Growth-Hormone Secretion Patterns on Regulation of Gender-Divergent Expression of Oat5, Urat1, and Oatp4c1 in Kidneys of Mice. Growth hormone secretion is different between male and female animals. Growth hormone is secreted in a pulsatile pattern in male animals but in a continuous pattern in females. Regulatory mechanisms for the gender-dimorphic expression of Oat5, Urat1, and Oatp4c1 transporters by growth hormone secretion patterns were further determined in kidneys of mice (Fig. 8).

In WT mice, Oat5 mRNA expression is female-predominant in mouse kidneys. Disruption of GH function, as depicted in the *lit/lit* mice, markedly decreased constitutive expression of Oat5 and diminished gender-predominant Oat5 mRNA expression. However, GH administration to *lit/lit* mice did not alter kidney Oat5 mRNA expression. Therefore, GH had no effect on constitutive or gender-divergent Oat5 mRNA expression in mouse kidney.

In WT mice, Urat1 mRNA expression is male-predominant. Urat1 mRNA expression in the *lit/lit* mice is also male-predominant. However, GH administration to *lit/lit* mice did not alter kidney Urat1 mRNA expression (Fig. 8).

In WT mice, Oatp4c1 mRNA expression is male-predominant in mouse kidneys. Disruption of GH signaling did not alter constitutive Oatp4c1 mRNA expression in kidneys of mice. In *lit/lit* mice, male-pattern GH administration increased Oatp4c1 mRNA levels in female

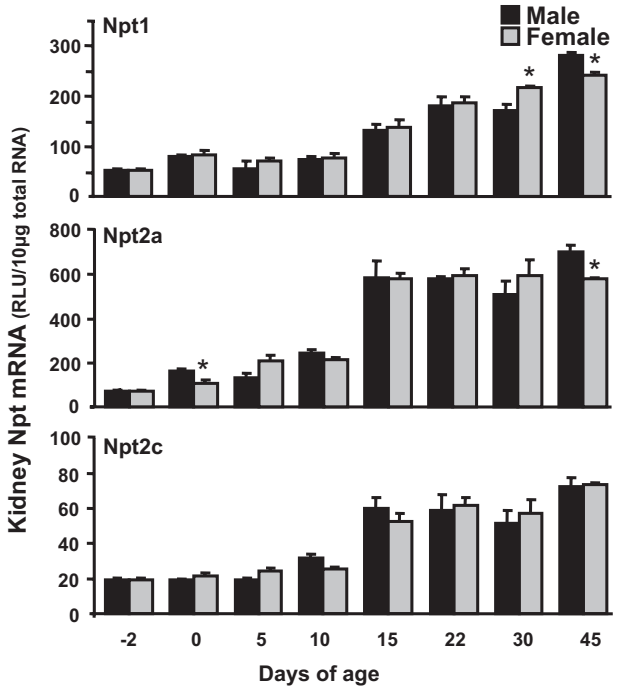

FIG. 4. Ontogenic expression of Npt1, 2a, and 2c mRNA in mouse kidneys. Total RNA from C57BL/6 mice of each age (n = 5/gender/age) was analyzed by the bDNA assay. Data are presented as mean ± S.E.M. *, statistically significant difference between male and female mice ($p < 0.05$). RLU, relative light units.

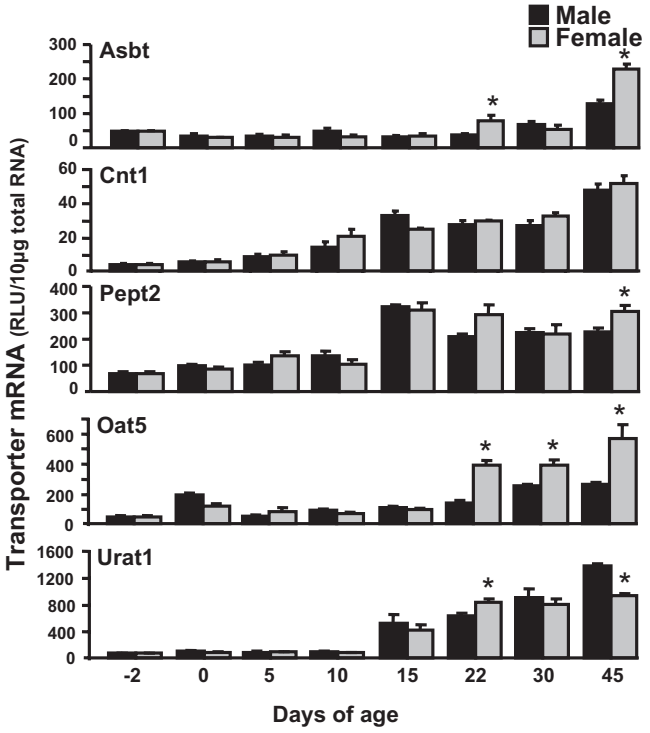

FIG. 5. Ontogenic expression of Asbt, Cnt1, Pept2, Oat5, and Urat1 mRNA in mouse kidneys. Total RNA from C57BL/6 mice of each age (n = 5/gender/age) was analyzed by the bDNA assay. Data are presented as mean ± S.E.M. *, statistically significant difference between male and female mice ($p < 0.05$). RLU, relative light units.

mice, but not in male mice, leading to disseemance of gender-predominant mRNA expression of Oatp4c1. In contrast, female-pattern GH administration to *lit/lit* mice did not alter Oatp4c1 mRNA expression (Fig. 8).

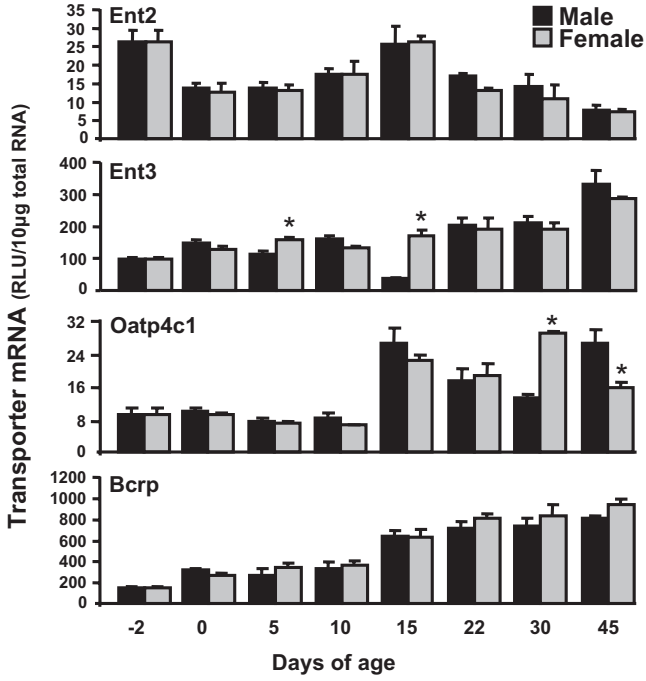

FIG. 6. Ontogenic expression of Ent2, Ent3, Oatp4c1, and Bcrp mRNA in mouse kidneys. Total RNA from C57BL/6 mice of each age (n = 5/gender/age) was analyzed by the bDNA assay. Data are presented as mean ± S.E.M. *, statistically significant difference between male and female mice ($p < 0.05$). RLU, relative light units.

Discussion

The tissue distribution and ontogeny of some transporters, which exist in kidneys of mice as highlighted in Fig. 1, were determined in the present study. In addition to expression in mouse kidneys, some of these transporters are also highly expressed in other tissues. For example, Npt2b is also expressed in liver and lung, Npt2c in liver and colon, Abca1 in placenta and ovary, and Asbt in ileum (Figs. 2 and 3).

The kidneys of newborn animals are functionally immature. The renal clearance of *p*-aminohippuric acid, an organic acid, is low in young humans (Calcagno and Rubin, 1963), dogs (Horster and Valtin, 1971), rats (Horster and Lewy, 1970), rabbits, and sheep (Phelps et al., 1976). The renal transport of tetraethylammonium, an organic base, is also underdeveloped in newborns (Rennick et al., 1961). Because *p*-aminohippuric acid and tetraethylammonium are transported by proximal tubule cells into the filtrate, the decrease in renal clearance of these chemicals in newborns indicates that both organic acid and base transporters are immature (Rennick et al., 1961). The present and previous studies showed that the developmental patterns of transporters are responsible for the inability of immature kidneys to excrete many chemicals. Oat1–3 (Buist and Klaassen, 2004), Oct1 and 2, Octn1 and n2 (Alnouti et al., 2006), Oatp1a1, 1a6, and 3a1 (Cheng et al., 2005), and Mrp2–4 (Maher et al., 2005), combined with the currently studied transporters (Npt1, 2a, 2c, Asbt, Cnt1, Pept2, Oat5, Urat1, Ent3, Oatp4c1, and Bcrp) are all lowly expressed in mice before 15 days of age (Table 2). However, there are some transporters in kidneys that are expressed at an early age, such as Ent2 (Fig. 6; Table 2). In addition to Ent2, Oatp1a4, 2a1, 2b1, and 3a1 (Cheng et al., 2005), as well as Mrp1, 5, and 6 (Maher et al., 2005), are also expressed early, and may be responsible for the adult rate of urinary excretion and reuptake of some chemicals at birth. However, because of low expression of a majority of the renal transporters, renal function, in general, of newborns is immature.

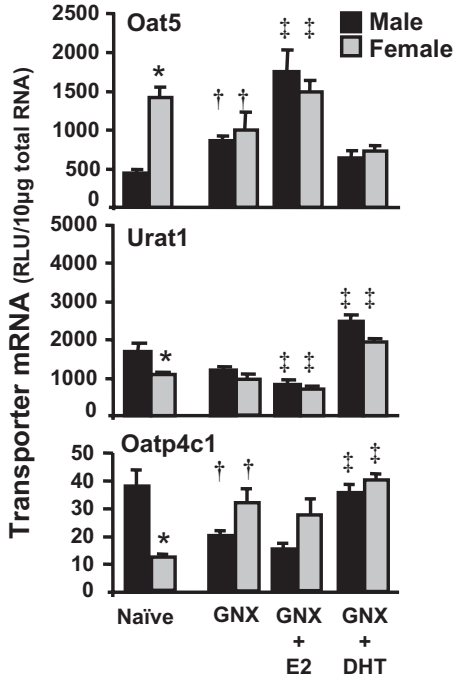

FIG. 7. Effects of sex hormones on the gender-divergent mRNA expression of Oat5, Urat1, and Oatp4c1 in kidneys of naive and gonadectomized (GNX) mice. Total kidney RNA was isolated and analyzed by the bDNA signal amplification assay for mRNA expression of each transporter. The data are presented as mean ± S.E.M. (n = 6–7/group). The treatments were separated into groups: GNX (placebo administered to gonadectomized mice), GNX + DHT (5α-dihydroxytestosterone administered to gonadectomized mice), and GNX + E2 (17β-estradiol administered to gonadectomized mice). ∗, statistical difference ($p < 0.05$) between male and female mice; †, statistically significant difference ($p < 0.05$) between naive mice and the same gender, placebo-treated gonadectomized mice; ‡, statistically significant difference ($p < 0.05$) between placebo-treated gonadectomized mice and the same gender gonadectomized mice after sex hormone replacement.

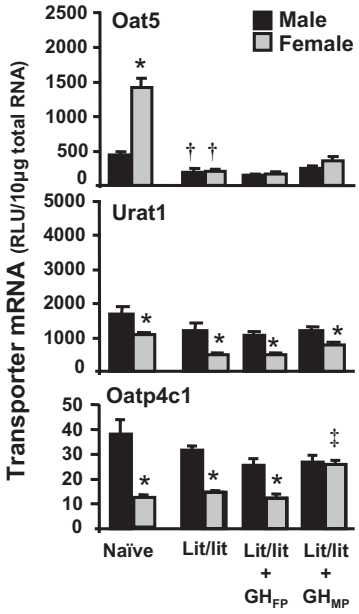

FIG. 8. Effects of growth hormone on the gender-divergent mRNA expression of Oat5, Urat1, and Oatp4c1 in kidneys of naive and *lit/lit* mice. Total kidney RNA was isolated and analyzed by the bDNA signal amplification assay for mRNA expression of each transporter. The data are presented as mean ± S.E.M. (n = 6–7/group). The treatments were separated into groups: Lit (placebo administered to *lit/lit* mice), Lit + GH_{MP} (rat GH twice daily administered by intraperitoneal injection to *lit/lit* mice mimicking male-pattern GH secretion), and Lit + GH_{FP} (continuous infusion to *lit/lit* mice via a subcutaneous implanted 21-day-release 1-mg rat GH pellet mimicking female-pattern GH secretion). ∗, statistical difference ($p < 0.05$) between male and female mice; †, statistically significant difference ($p < 0.05$) between naive mice and the same gender, placebo-treated *lit/lit* mice; ‡, statistically significant difference ($p < 0.05$) between placebo-treated *lit/lit* mice and the same gender *lit/lit* mice after growth hormone replacement.

Gender differences in the expression of a number of transporters in the kidneys of mice are noted. For example, male-predominant Npt1, Npt2a, Urat1, and Oatp4c1, as well as female-predominant Asbt, Oat5, and Pept2 were observed in kidneys of mice. Gender differences in transporter gene expression may be the result of regulation by sex hormones and/or gender-dimorphic GH secretory patterns. Androgens and estrogens alter gene expression by directly stimulating gene transcription or stabilizing the mRNA of genes (Beato, 1989; Paul et al., 1990; Kimura et al., 1994). Growth hormone is also an important regulator of gender-divergent gene expression. Gender-divergent secretion patterns of GH lead to differential patterns in gene expression. By using gonadectomized and *lit/lit* mouse models, gender-divergent regulation of Oat5, Urat1, and Oatp4c1 in mouse kidneys was shown to be mainly controlled by sex hormones (Fig. 7) but not by GH (Fig. 8). Consistent with previous studies (Cheng et al., 2006; Maher et al., 2006), gender differences of transporters in kidneys are mainly determined by sex hormones. For example, male-predominant Oatps (Oatp1a1 and 3a1) and female-predominant Mrp3 in mouse kidneys are due to stimulatory effect of androgens and estrogens, respectively (Cheng et al., 2006; Maher et al., 2006).

One interesting phenomenon is that gender-divergent expression patterns of some transporters, such as Npt1, Oatp4c1, and Urat1, are age-related (Figs. 5–7). For example, Npt1, Oatp4c1, and Urat1 are all male-predominant in mouse kidneys at 45 days of age but are female-predominant at certain early ages (30 days of age for Npt1 and Oatp4c1 and 22 days of age for Urat1). The underlying regulatory

TABLE 2

Developmental patterns of transporters in mouse kidneys

	Transporter	Fetal (Day −2)	Birth (Day 0)	Early (Days 5–10)	Middle (Days 15–23)	Late (Day 30→)
Renal uptake from blood	Oatp4c1				X[a]	
Renal uptake from filtrate	Cnt1				X	
	Npt1				X	
	Npt2a				X	
	Npt2c				X	
	Pept2				X	
	Oat5				X	
	Urat1				X	
	Asbt				X	X
Renal efflux into filtrate	Bcrp				X	
Renal efflux into blood	Ent2	X			X	
	Ent3				X	

[a] X, indicates the age when the mRNA expression of the transporter approaches its highest level.

mechanism is unknown. The present study and other studies showed that gender-divergent gene expression in mouse kidneys is mainly due to either androgens or estrogens. Therefore, the age-related gender-divergent switch of Npt1, Oatp4c1, and Urat1 in mouse kidneys is probably due to effects of sex hormones.

Among the transporters examined in this study, Npt1, 2a, and 2c are highly expressed in mouse kidneys, in accordance with previous reports (Tenenhouse et al., 1998; Segawa et al., 2002). In contrast, Npt2b is expressed in mouse liver, lung, and brain (Fig. 3). A previous report showed that Npt2b is expressed in the small intestine of mice but not in kidney and is responsible for apical intestinal sodium-P_i cotransport (Hilfiker et al., 1998).

The data described in the present study provide information regarding the transporters in kidneys and indicate that many of the transporters involved in transport of chemicals into and out of proximal tubular cells have developmental and gender-specific mRNA expression differences in mouse kidneys. The implication of these observations is that age- and gender-related differences in pharmacokinetics of chemicals may in part be ascribed to differences in transporter expression in mouse kidneys. Therefore, when one is attempting to explain age- or gender-related discrepancies in xenobiotic pharmacokinetics, functional studies of transporters that display variations in expression should be considered to exclude or confirm a contribution of transporter expression patterns to the observed differences in pharmacokinetics.

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