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Tissue Distribution and Ontogeny of Organic Cation Transporters in Mice

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Abbreviations: Octs: organic cation transporters, EMT: extraneuronal monoamine transporter, TEA: trimethylamine, bDNA: branched DNA signal amplification assay, E2: 17β-estradiol, DHT: 5α-dihydroxytestosterone, SCD: carnitine deficiency syndrome, RLU: relative light unit(s).

Abstract

Organic cation transporters (Octs) play an important role in transporting cationic xeno- and endobiotics across biological membranes. Little is known about Octs in mice, therefore the tissue distribution and developmental changes in the mRNA expression of Octs in mice were quantified. Oct1, Oct2, Oct3, Octn1, Octn2, and Octn3 mRNA expression was quantified in 14 tissues from male and female mice using the branched DNA signal amplification assay (bDNA). Oct1 mRNA expression was highest in kidney, followed by liver. Oct2 mRNA was almost exclusively expressed in kidney, with male mice having twice that in female mice. The higher expression of Oct2 in male mice is due to testosterone. Oct3 mRNA was most highly expressed in placenta, ovary, and uterus, but was expressed at low levels in most tissues. Octn1 and Octn2 mRNA expression was similar, with highest levels in kidney followed by small intestine. Octn3 mRNA was almost exclusively expressed in testes. The developmental expression of Oct1, Oct2, Octn1, and Octn2 mRNA in kidneys as well as Oct1 in livers was determined in young mice. Ontogenic expression data indicate that each of the Oct transporters approached adult expression levels by about three weeks of age. The gender difference in Oct mRNA expression did not become apparent until day 30 after birth. The differences in tissue distribution of the Octs may play an important role in drug disposition to various tissues. Furthermore, low expression of the Octs in young animals may affect the pharmacokinetic behavior of drugs compared to that in adults.

Introduction

Living organisms have several mechanisms to eliminate xenobiotics and endogenous substances as a defense mechanism against the harmful effects inflicted by these substances. Cationic substances are of special interest because they comprise around 50% of the available therapeutic chemicals (Pritchard and Miller, 1993; Oude Elferink et al., 1995). Specific transport systems in cells are responsible for transferring these water-soluble substances through the lipophilic biological membranes in order to exert pharmacological action or to facilitate elimination.

In recent years, several organic cation transporters (Octs), belonging to the SLC22 gene family, have been cloned and characterized from several species. These include Oct1, Oct2, Oct3, Octn1, Octn2, and Octn3. The first organic cation transporter (Oct1) was cloned from rat kidney in 1994 (Grundemann et al., 1994). Subsequently, Oct2 and Oct3, two organic cation transporters with high homology to Oct1, were cloned and characterized in humans, rats, mice, and rabbits (Okuda et al., 1996; Gorboulev et al., 1997; Zhang et al., 1997; Kekuda et al., 1998; Urakami et al., 1998; Green et al., 1999; Karbach et al., 2000).

Cellular localization studies in kidney, using in situ hybridization, immunohistochemistry, western and northern blotting, have concluded that Oct1 is localized in the basolateral membrane of S1 and S2 segments of renal proximal tubules. Oct1 expression was also assigned to the sinusoidal membrane of hepatocytes in rat liver (Meyer-Wentrup et al., 1998). Oct2 is also localized to the basolateral membranes, but of the S2 and S3 rather than S1 and S2 segments of the renal proximal tubules (Urakami et al., 1998; Karbach et al., 2000).

The expression and function of Octs are regulated by gender and different diseases. Oct2 expression is gender biased in rats, in favor of males (Urakami et al., 1999; Slitt et al., 2002).

Oct2 expression is down-regulated to female levels in gonadectomized or estradiol-treated male rats. Also, Oct2 expression is up-regulated several fold in female rats treated with testosterone (Urakami et al., 2000; Slitt et al., 2002). Different pathological conditions also impact the regulation of Octs. Diabetic rats undergo down-regulation of Oct1 and Oct2 renal expression, which is reversed by insulin administration (Grover et al., 2004). Chronic renal failure in rats produced by 5/6 nephrectomy, results in down-regulation of Oct2 in rats, which is reversed by testosterone administration (Ji et al., 2002).

Oct3 is also called EMT (extraneuronal monoamine transporter) for its role in the extraneuronal monoamine uptake system (uptake2) (Grundemann et al., 1998; Wu et al., 1998a). The uptake2 system participates in the uptake of extracellular monoamines in peripheral tissues and glia cells. Inhibition of the uptake2 system and therefore Oct3 is a potential pharmacological target for the treatment of depression by increasing monoamine levels in the CNS (Schildkraut and Mooney, 2004).

Octn1-Octn3 are referred to as carnitine organic cation transporters and have a low degree of similarity, in the range of 30-35%, to the Oct1-3 genes. Model organic cations like trimethylamine (TEA) as well as carnitine are substrates for Octn1-3 (Yabuuchi et al., 1999). Oct carnitine transport specificity can be described as the ratio of their TEA: carnitine uptake (Tamai et al., 2000). Octn3 is the most carnitine selective transporter, whereas Octn1 is the least selective. Octn1 and Octn2 transport carnitine and its acyl derivatives in a Na^+-dependent manner (Wu et al., 1999). Therefore, Octns represent a unique case where various substrates are transported by different mechanisms, suggesting multiple binding sites in the protein (Wu et al., 1998a; Seth et al., 1999).

In the present study, the tissue distribution, gender differences and ontogenic expression of Oct1, 2, 3, Octn1, n2, and n3 in mice were determined. Most studies in the literature concerning the tissue distribution of Octs have been limited to a few tissues, and were not quantitative. These previous studies have primarily been performed in rat and human tissues, with limited data available for mice. Therefore, in the present study, the relative distribution of Oct mRNAs as well as the developmental changes in Octs in both male and female mice were evaluated. Understanding the tissue-specific expression patterns of Octs may help determine their contribution in the distribution of xeno- and endobiotics to various tissues. Furthermore, understanding gender differences and ontogeny of Octs expression may help determine the molecular basis for differences in drug disposition between male vs. females, and children vs. adults, respectively.

Materials and Methods

Reagents. Pellets for subcutaneous release of the hormones used in this study, 5α-dihydroxytestosterone (5 mg; 21-day release) and 17β-estradiol (0.5 mg; 21-day release) were formulated by Innovative Research of America (Sarasota, FL) to deliver slightly higher than normal circulating levels of these hormones (i.e. estrogen levels of ~750-1500 pg/ml).

Animals. Eight-weeks-old male and female C57BL/6 mice were purchased from Charles River Laboratories Inc (Wilmington, MA). Animals were housed in a temperature-, light-, and humidity-contolled environment. Mice were fed Laboratory Rodent Chow W (Harlan Teklad, Madison, WI) ad libitum. Tissues were removed from five mice of each gender, frozen in liquid nitrogen, and stored at -80ºC until mRNA isolation. For the ontogeny study, livers and kidneys from male and female mice were collected at -2, 0, 5, 10, 15, 22, 30, and 45 days of age (n= 5/gender/age). Male and female pups were pooled together at age -2 because it was difficult to differentiate their gender.

Gonadectomy and Sex Hormone replacement. Mice were castrated or ovariectomized at 37 days of age by Charles River Laboratories. At 54 days of age, 5α-dihydroxytestosterone (5 mg) or 17β-estradiol (0.5 mg) 21-day time-release pellets (Innovative Research of America, Sarasota, FL) were subcutaneously implanted interscapularly in intact and gonadectomized male and female mice under isofluorane anesthesia. The mice were separated into five treatment groups, with six mice/gender/treatment: (1) intact animals + placebo, (2) castration + 5α-dihydroxytestosterone, (3) castration + 17β-estradiol, (4) ovariectomy + 5α-dihydroxytestosterone, and (5) ovariectomy + 17β-estradiol. Kidneys were removed at 64 days of age from gonadectomized and age-matched intact control mice.

Total RNA Isolation. Total RNA was isolated using RNA-Bee reagent (Tel-Test, Inc., Friendswood, TX) according to the manufacturer's protocol. Total RNA concentrations were determined spectrophotometrically at 260 nm. One μg/μl solutions were prepared from the stock RNA solution by dilution with diethyl pyrocarbonate-treated deionized water. Integrity of RNA samples was evaluated visually using agarose gel electrophoresis. Samples were then visualized under ultraviolet light by ethidium bromide fluorescence.

Branched DNA Signal Amplification (bDNA) Analysis. The mRNA of each Oct in mouse tissues was quantified using the bDNA assay (Quantigene® bDNA signal amplification kit; Bayer Corp., Emeryville, CA) with modifications (Hartley and Klaassen, 2000). Gene sequences of interest were accessed from GenBank. Target sequences were analyzed using ProbeDesigner software v1.0 (Bayer Corp., Emerville, CA) to design oligonucleotide probe sets (capture, label, and blocker probes). All probes were designed with a melting temperature of 63ºC, enabling hybridization conditions to be held constant (i.e., 53ºC) during each hybridization step. Each developed probe was submitted to the National Center of Biotechnology Information (NCBI, Bethesda, MD) by basic local alignment search tool (BLASTn) to ensure minimal cross-reactivity with other known mouse sequences. Oligonucleotides with a high degree of similarity (> 80%) to other mouse gene transcripts were eliminated from the design. The probe sets sequences and functions are listed in Table 1.

Total RNA (1 μg/μl; 10 μl/well) was added to each well of a 96-well plate containing 50 μl of each diluted probe set. RNA was allowed to hybridize with the probe sets overnight at 53 ºC. Subsequent hybridization steps were carried out according to the manufacturer's protocol, and luminescence was quantified with a Quantiplex 320 bDNA luminometer interfaced with

Quantiplex Data Management software v5.02. Data are presented as relative light units (RLU) per 10 μg of total RNA.

Statistical Analysis. Gender differences in mice were determined using student's *t* test with significance set at $p \leq 0.05$. Bars represent mean ± SEM.

Results

Tissue Distribution of Octs mRNA. Fourteen tissues were analyzed for Oct1, Oct2, Oct3, Octn1, Octn2, and Octn3 mRNA. These tissues include liver, kidney, lung, stomach, duodenum, jejunum, ileum, large intestine, heart, brain, gonads (testes and ovaries), placenta, and uterus. mRNA levels for each gene and in each tissue are reported as the average of data obtained from 5 C57BL/6 mice.

Oct1 mRNA levels were highest in kidney followed by liver (Figure 1). Oct1 expression in liver was about half of that in kidney. Oct1 mRNA was moderately expressed in the small and large intestine (10% of the kidney levels). Gender-specific differences were found in Oct2 mRNA expression; Oct2 kidney mRNA levels in male mice were twice that in female mice. Oct3 mRNA levels were low in most tissues, except the female reproductive tissues, such as placenta, ovary, and uterus (Figure 1).

Octn1 was ubiquitously expressed in most tissues with highest expression in kidney, followed by small intestine (Figure 2). Octn2 mRNA tissue distribution pattern was quite similar to Octn1. Octn3 was almost exclusively expressed in testes. The mRNA levels in the female ovaries were about 15% of that in the male testes (Figure 2). Summary of mouse organs with predominant Octs mRNA expression is presented in table 2.

Ontogeny of Oct1, Oct2, Octn1 and Octn2. Because kidney had the highest Oct mRNA expression, this tissue was selected to study the developmental changes in the mRNA expression of Oct1, Oct2, Octn1 and Octn2. Oct1 ontogeny was also studied in liver because, unlike other Octs, Oct1 mRNA was also abundant in liver. mRNA levels were quantified in tissues from 5 male and female mice at ages of -2, 0, 5, 10, 15, 22, 30 and 45 days.

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Oct1 mRNA expression was very low 2 days before birth, but gradually increased during the first three weeks of age, reaching a plateau around day 22 in both kidney (Figure 3) and liver (Figure 4) . At 45 days of age, renal and hepatic mRNA levels of Oct1 were 4-6 times higher than immediately after birth. Generally, there were no gender differences in Oct1 mRNA expression except for day 0 and day 22, where male mice have predominant expression in kidneys and livers, respectively. Oct2 mRNA levels were also essentially undetectable two days before birth, but were about one-fourth of adult levels at birth. Interestingly, the gender differences in mRNA expression of Oct2 did not appear until 30 days after birth, when the female levels reached a plateau and the male levels continued to increase (Figure 3). Octn1 and Octn2 mRNA expression gradually increased from 2 days before birth until about 2 weeks of age, when the levels reached a plateau (Figure 3).

Renal OCT2 Expression in Gonadectomized Mice. To determine the mechanism of the gender difference in Oct2 mRNA expression, studies were performed to determine the effect of gonadectomy, estradiol (E2), and dihydrotesosterone (DHT) on OCT2 expression in kidneys from male and female mice. Consistent with the tissue distribution data, OCT2 mRNA levels in kidneys of adult male mice were higher than in female mice (Fig 5). In gonadectomized male mice, OCT2 mRNA levels decreased approximately 50%, similar to levels in female kidneys. Oct2 expression in kidney was not affected by gonadectomy in female mice. E2 had no effect on Oct2 expression in either male or female gonadectomized mice. However, DHT administration to gonadectomized animals increased Oct2 expression in both genders. mRNA levels of Oct2 were restored to that present in intact male mice (Fig 5).

Discussion

Tissue distribution of 6 organic cation transporters in mice, namely Oct1-3 and Octn1-3, were examined in this study. Most studies in the literature concerning the tissue distribution of Octs have been limited to a few tissues, and were not quantitative. These previous studies have primarily been performed with rat and human tissues, with limited data available for mice. The mouse is becoming a more common laboratory species because of the availability of the mouse genome sequence as well as transgenic and gene-knockout mice. Therefore, in the present study we evaluated the relative distribution of the mRNA of Octs in 14 tissues in mice.

Data from this study demonstrate that Oct1 mRNA expression is highest in kidney and liver, followed by the small intestine (Figure 1). These results agree with previous reports of the mRNA distribution of Octs in rats (Grundemann et al., 1994; Grundemann et al., 1997; Urakami et al., 1998; Wu et al., 1998a; Slitt et al., 2002). In humans, Oct1 mRNA appears to be expressed in liver but not in kidney (Gorboulev et al., 1997; Zhang et al., 1997). This represents a major difference in Oct1 tissue distribution between rodents and humans. The lack of Oct1 expression in human kidney might cause major differences in the pharmacokinetics of Oct substrates compared to rodents. The developmental expression of Oct1 was evaluated in kidneys and livers because these tissues have the highest Oct1 mRNA expression levels. Oct1 mRNA expression in both kidney and liver gradually increased from 2 days before birth to 22 days after birth and remained relatively constant thereafter (Figures 3 and 4).

The present study indicates that kidney is the organ in mice with the predominant expression of Oct2. This finding is consistent with previous data from rats (Okuda et al., 1996; Grundemann et al., 1997; Urakami et al., 1998; Wu et al., 1998a; Urakami et al., 1999) and humans (Gorboulev et al., 1997). In contrast to Oct1, the tissue distribution of Oct2 is similar

between all 3 species. Oct2 mRNA expression is gender dependent; the expression of Oct2 mRNA in female mice is approximately half of that in males (Figure 1). This gender difference is not evident until the mice are three weeks of age (Figure 3). In gonadectomized mice, expression of Oct2 mRNA in males was down regulated to levels detected in female mice. DHT administration to gonadectomized animals restored the male mRNA expression to the same level as in intact animals and also up regulated the female mRNA expression (Figure 5). Together these data indicate that male sex hormones are responsible for the higher expression of Oct2 in male mice. The role of sex hormones in regulating Oct2 expression has been examined in rats. Oct2 expression was suppressed by estrogens, and enhanced by androgens, which results in a gender bias in Oct2 expression in favor of males (Urakami et al., 1999; Urakami et al., 2000; Slitt et al., 2002).

Oct3 mRNA was expressed at low levels in most tissues of mice. The highest expression of Oct3 mRNA was found in placenta and ovaries, whereas lower levels were detected in uterus, heart, lung, brain, and ileum (Figure 1). This high expression of Oct3 in placenta, and its widespread expression at low levels in many tissues is in agreement with previous data in rats (Kekuda et al., 1998; Wu et al., 1998a; Slitt et al., 2002), mice (Verhaagh et al., 1999) and humans (Verhaagh et al., 1999; Wu et al., 2000b). This widespread expression of Oct3 supports its role for the non-neuronal uptake of monoamine neurotransmitters in both the CNS and peripheral tissues. Similar to Oct2, Oct3 tissue distribution has similar pattern across rodents (mice and rats) and humans.

Oct1-3 share high similarity in their cDNA nucleotide sequence and their predicted amino acid sequence. When Octn1 was cloned, it was annotated differently (Octn instead of Oct) due to the low homology in amino acid sequence with the known members of Octs1-3 (30%

similarity). Also, Octn1 is functionally different by being pH-dependent rather than potential dependent, as are Oct1-3. In the present study, Octn1 mRNA was ubiquitously expressed in most tissues; the only tissue with essentially no expression was the liver (Figure 2). The highest expression of Octn1 was in kidney, small intestine, and heart. In rats, Octn1 mRNA had a similar tissue distribution (Wu et al., 2000a; Slitt et al., 2002). However, another study reported high expression of Octn1 mRNA in liver, besides kidney and small intestine in rats (Wu et al., 2000a). In humans, Octn1 mRNA has been reported to be highly expressed in kidney and bone marrow, whereas it is moderately expressed in skeletal muscle, lung, and placenta, has low expression in small intestine, heart, spleen, and uterus, and undetected in liver (Tamai et al., 1997). Despite the differences in Octn1 tissue distribution between the 3 species, it appears that Octn1 is most highly expressed in kidney and intestines of all species, but expressed at low levels in most other tissues.

Like Octn1, Octn2 transports organic cations in a Na^+-independent manner. In contrast, Octn2 transports carnitine in a Na^+-dependent manner (Ohashi et al., 1999; Wu et al., 1999). Octn2 mRNA expression in mice is high in kidney, moderate in small intestine, and present at low levels in other tissues. The tissue distribution of Octn2 in mice is similar to that for rats (Slitt et al., 2002). A previous study in mice reported high and equivalent Octn2 mRNA expression in testis, kidney, and liver, and low expression in heart (Tamai et al., 2000). In contrast, western blot analysis in the same study indicated the highest Octn2 protein in kidney, moderate in liver and heart, and lowest in testes. In humans, Octn2 is highly expressed in kidney, skeletal muscles, placenta, heart, and pancreas, with no expression in liver (Wu et al., 1998b; Tamai et al., 2000). There are major differences in the Octn2 tissue distribution patterns between

the 3 species, however, Octn2 appears to be primarily expressed in kidney in the three species: rats, mice, and humans.

Octn2 is thought to play a major role in the high affinity Na^+-dependent (Wu et al., 1999) carnitine uptake. Certain mutations (Tang et al., 1999) in the Octn2 gene results in premature stop codons, and consequently the mutant gene produces truncated proteins that causes primary systemic carnitine deficiency syndrome (SCD) (Inano et al., 2003), which is associated with cardiomyopathy, skeletal muscle myopathy, and hypoglycemia (Tamai et al., 2000). SCD symptoms are treated with L-carnitine supplementation, despite the defect in the Octn2 transporter (Horiuchi et al., 1993; Tein, 2003). Therefore, alternative pathways are speculated to compensate for the defect in Octn2 (Tamai et al., 2000). Carnitine administration might also up-regulate the expression of these alternative carnitine transporters. A mutant mouse strain that exhibits a SCD phenotype (jvs), also has a mutated Octn2 and a functional loss of Octn2 (Koizumi et al., 1988).

Octn3 mRNA expression was highest in testes of mice; low levels were detected in ovary, ileum, and jejunum. The almost exclusive expression of Octn3 in testis has also been reported for humans (Enomoto et al., 2002). In mice, Octn3 protein expression is primarily found in testis, and very weakly expressed in kidney (Tamai et al., 2000). The present data suggests that Octn3 is predominantly expressed in testes. Therefore, Octn3 appears to be responsible for the uptake of carnitine into testes, and eventually into sperm. Octn3 has a higher selectivity for carnitine transport than Octn1 or Octn2; Octn3 has little or no affinity for organic cation model compounds, i.e. TEA, and is the only Octn to transport carnitine in a Na^+-independent manner. Therefore Octn3 is distinct from Octn1 and Octn2 in both tissue distribution and substrate specificity. Carnitine is important for the maturation of spermatozoa into fertile and motile

sperm (Jeulin and Lewin, 1996; Enomoto et al., 2002). Clinical administration of carnitine results in an increase in sperm number and motility (Enomoto et al., 2002). Therefore, Octn3 is indeed a potential target for male infertility screening and treatment. Mutations in Octn3 have also been suggested to represent a risk factor in inflammatory bowel diseases, which may be responsive to carnitine therapy (Lamhonwah et al., 2003).

In summary, the present data demonstrate the relative tissue distribution of Oct1-3 and Octn1-3 mRNA in 14 tissues of mice. Oct mRNA were expressed highest in kidney, except for Oct3, which was mainly expressed in placenta and female sex organs, and Octn3, which was primarily expressed in testes. Oct1 was mainly expressed in kidney followed by liver. Oct2 was almost exclusively expressed in kidney. Oct2 expression was higher in male than female kidneys, due to its induction by testosterone. Oct3 was mainly expressed in placenta and uterus. Octn1 was mainly expressed in kidney, intestine, and heart. Octn2 was mainly expressed in kidney and intestine. Octn3 was almost exclusively expressed in testes. With the exception of Oct1, Octs show similar tissue distribution patterns between mice, rats, and humans. Finally, all Octs reached adult mRNA expression levels at 3-4 weeks after their birth.

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References

Enomoto A, Wempe MF, Tsuchida H, Shin HJ, Cha SH, Anzai N, Goto A, Sakamoto A, Niwa T, Kanai Y, Anders MW and Endou H (2002) Molecular identification of a novel carnitine transporter specific to human testis. Insights into the mechanism of carnitine recognition. *J Biol Chem* **277:**36262-36271.

Gorboulev V, Ulzheimer JC, Akhoundova A, Ulzheimer-Teuber I, Karbach U, Quester S, Baumann C, Lang F, Busch AE and Koepsell H (1997) Cloning and characterization of two human polyspecific organic cation transporters. *DNA Cell Biol* **16:**871-881.

Green RM, Lo K, Sterritt C and Beier DR (1999) Cloning and functional expression of a mouse liver organic cation transporter. *Hepatology* **29:**1556-1562.

Grover B, Buckley D, Buckley AR and Cacini W (2004) Reduced expression of organic cation transporters rOCT1 and rOCT2 in experimental diabetes. *J Pharmacol Exp Ther* **308:**949-956.

Grundemann D, Babin-Ebell J, Martel F, Ording N, Schmidt A and Schomig E (1997) Primary structure and functional expression of the apical organic cation transporter from kidney epithelial LLC-PK1 cells. *J Biol Chem* **272:**10408-10413.

Grundemann D, Gorboulev V, Gambaryan S, Veyhl M and Koepsell H (1994) Drug excretion mediated by a new prototype of polyspecific transporter. *Nature* **372:**549-552.

Grundemann D, Schechinger B, Rappold GA and Schomig E (1998) Molecular identification of the corticosterone-sensitive extraneuronal catecholamine transporter. *Nat Neurosci* **1:**349-351.

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.

Horiuchi M, Yoshida H, Kobayashi K, Kuriwaki K, Yoshimine K, Tomomura M, Koizumi T, Nikaido H, Hayakawa J and Kuwajima M (1993) Cardiac hypertrophy in juvenile visceral steatosis (jvs) mice with systemic carnitine deficiency. *FEBS Lett* **326:**267-271.

Inano A, Sai Y, Nikaido H, Hasimoto N, Asano M, Tsuji A and Tamai I (2003) Acetyl-L-carnitine permeability across the blood-brain barrier and involvement of carnitine transporter OCTN2. *Biopharm Drug Dispos* **24:**357-365.

Jeulin C and Lewin LM (1996) Role of free L-carnitine and acetyl-L-carnitine in post-gonadal maturation of mammalian spermatozoa. *Hum Reprod Update* **2:**87-102.

Ji L, Masuda S, Saito H and Inui K (2002) Down-regulation of rat organic cation transporter rOCT2 by 5/6 nephrectomy. *Kidney Int* **62:**514-524.

Karbach U, Kricke J, Meyer-Wentrup F, Gorboulev V, Volk C, Loffing-Cueni D, Kaissling B, Bachmann S and Koepsell H (2000) Localization of organic cation transporters OCT1 and OCT2 in rat kidney. *Am J Physiol Renal Physiol* **279:**F679-687.

Kekuda R, Prasad PD, Wu X, Wang H, Fei YJ, Leibach FH and Ganapathy V (1998) Cloning and functional characterization of a potential-sensitive, polyspecific organic cation transporter (OCT3) most abundantly expressed in placenta. *J Biol Chem* **273:**15971-15979.

Koizumi T, Nikaido H, Hayakawa J, Nonomura A and Yoneda T (1988) Infantile disease with microvesicular fatty infiltration of viscera spontaneously occurring in the C3H-H-2(0) strain of mouse with similarities to Reye's syndrome. *Lab Anim* **22:**83-87.

Lamhonwah AM, Skaug J, Scherer SW and Tein I (2003) A third human carnitine/organic cation transporter (OCTN3) as a candidate for the 5q31 Crohn's disease locus (IBD5). *Biochem Biophys Res Commun* **301:**98-101.

Meyer-Wentrup F, Karbach U, Gorboulev V, Arndt P and Koepsell H (1998) Membrane localization of the electrogenic cation transporter rOCT1 in rat liver. *Biochem Biophys Res Commun* **248:**673-678.

Ohashi R, Tamai I, Yabuuchi H, Nezu JI, Oku A, Sai Y, Shimane M and Tsuji A (1999) Na(+)-dependent carnitine transport by organic cation transporter (OCTN2): its pharmacological and toxicological relevance. *J Pharmacol Exp Ther* **291:**778-784.

Okuda M, Saito H, Urakami Y, Takano M and Inui K (1996) cDNA cloning and functional expression of a novel rat kidney organic cation transporter, OCT2. *Biochem Biophys Res Commun* **224:**500-507.

Oude Elferink RP, Meijer DK, Kuipers F, Jansen PL, Groen AK and Groothuis GM (1995) Hepatobiliary secretion of organic compounds; molecular mechanisms of membrane transport. *Biochim Biophys Acta* **1241:**215-268.

Pritchard JB and Miller DS (1993) Mechanisms mediating renal secretion of organic anions and cations. *Physiol Rev* **73:**765-796.

Schildkraut JJ and Mooney JJ (2004) Toward a rapidly acting antidepressant: the normetanephrine and extraneuronal monoamine transporter (uptake 2) hypothesis. *Am J Psychiatry* **161:**909-911.

Seth P, Wu X, Huang W, Leibach FH and Ganapathy V (1999) Mutations in novel organic cation transporter (OCTN2), an organic cation/carnitine transporter, with differential effects on the organic cation transport function and the carnitine transport function. *J Biol Chem* **274:**33388-33392.

Slitt AL, Cherrington NJ, Hartley DP, Leazer TM and Klaassen CD (2002) Tissue distribution and renal developmental changes in rat organic cation transporter mRNA levels. *Drug Metab Dispos* **30:**212-219.

Tamai I, Ohashi R, Nezu JI, Sai Y, Kobayashi D, Oku A, Shimane M and Tsuji A (2000) Molecular and functional characterization of organic cation/carnitine transporter family in mice. *J Biol Chem* **275:**40064-40072.

Tamai I, Yabuuchi H, Nezu J, Sai Y, Oku A, Shimane M and Tsuji A (1997) Cloning and characterization of a novel human pH-dependent organic cation transporter, OCTN1. *FEBS Lett* **419:**107-111.

Tang NL, Ganapathy V, Wu X, Hui J, Seth P, Yuen PM, Wanders RJ, Fok TF and Hjelm NM (1999) Mutations of OCTN2, an organic cation/carnitine transporter, lead to deficient cellular carnitine uptake in primary carnitine deficiency. *Hum Mol Genet* **8:**655-660.

Tein I (2003) Carnitine transport: pathophysiology and metabolism of known molecular defects. *J Inherit Metab Dis* **26:**147-169.

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.

Urakami Y, Okuda M, Masuda S, Saito H and Inui KI (1998) Functional characteristics and membrane localization of rat multispecific organic cation transporters, OCT1 and OCT2, mediating tubular secretion of cationic drugs. *J Pharmacol Exp Ther* **287:**800-805.

Urakami Y, Okuda M, Saito H and Inui K (2000) Hormonal regulation of organic cation transporter OCT2 expression in rat kidney. *FEBS Lett* **473:**173-176.

Verhaagh S, Schweifer N, Barlow DP and Zwart R (1999) Cloning of the mouse and human solute carrier 22a3 (Slc22a3/SLC22A3) identifies a conserved cluster of three organic cation transporters on mouse chromosome 17 and human 6q26-q27. *Genomics* **55:**209-218.

Wu X, George RL, Huang W, Wang H, Conway SJ, Leibach FH and Ganapathy V (2000a) Structural and functional characteristics and tissue distribution pattern of rat OCTN1, an organic cation transporter, cloned from placenta. *Biochim Biophys Acta* **1466:**315-327.

Wu X, Huang W, Ganapathy ME, Wang H, Kekuda R, Conway SJ, Leibach FH and Ganapathy V (2000b) Structure, function, and regional distribution of the organic cation transporter OCT3 in the kidney. *Am J Physiol Renal Physiol* **279:**F449-458.

Wu X, Huang W, Prasad PD, Seth P, Rajan DP, Leibach FH, Chen J, Conway SJ and Ganapathy V (1999) Functional characteristics and tissue distribution pattern of organic cation transporter 2 (OCTN2), an organic cation/carnitine transporter. *J Pharmacol Exp Ther* **290:**1482-1492.

Wu X, Kekuda R, Huang W, Fei YJ, Leibach FH, Chen J, Conway SJ and Ganapathy V (1998a) Identity of the organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake2) and evidence for the expression of the transporter in the brain. *J Biol Chem* **273:**32776-32786.

Wu X, Prasad PD, Leibach FH and Ganapathy V (1998b) cDNA sequence, transport function, and genomic organization of human OCTN2, a new member of the organic cation transporter family. *Biochem Biophys Res Commun* **246:**589-595.

Yabuuchi H, Tamai I, Nezu J, Sakamoto K, Oku A, Shimane M, Sai Y and Tsuji A (1999) Novel membrane transporter OCTN1 mediates multispecific, bidirectional, and pH-dependent transport of organic cations. *J Pharmacol Exp Ther* **289:**768-773.

Zhang L, Dresser MJ, Gray AT, Yost SC, Terashita S and Giacomini KM (1997) Cloning and functional expression of a human liver organic cation transporter. *Mol Pharmacol* **51:**913-921.

Footnotes:

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Legends:

Figure 1: Total RNA was isolated from approximately 8-weeks-old male and female mice and analyzed by the bDNA signal amplification assay for Oct1, 2, and 3 mRNA expression. The data are presented as mean RLU ± SEM (n=5). * represents a statistically significant difference ($p \leq 0.05$) between males and females.

Figure 2: Total RNA was isolated from approximately 8-weeks-old male and female mice and analyzed by the bDNA signal amplification assay for Octn1, 2, and 3 mRNA expression. The data are presented as mean RLU ± SEM (n=5). * represents a statistically significant difference ($p \leq 0.05$) between males and females.

Figure 3: Total RNA was isolated from kidneys of -2, 0, 5, 10, 25, 30, and 45 day- old mice, and analyzed by bDNA signal amplification assay for Oct1, 2, Octn1, and 2 mRNA expression. The data are presented as mean RLU ± SEM (n=5). * represents a statistically significant difference ($p \leq 0.05$) between males and females.

Figure 4: Total RNA was isolated from livers of -2, 0, 5, 10, 25, 30, and 45 day-old mice, and analyzed by bDNA signal amplification assay for Oct1 mRNA expression. The data are presented as mean RLU ± SEM (n=5). * represents a statistically significant difference ($p \leq 0.05$) between males and females.

Figure 5: Total RNA was isolated from kidneys of wild type (intact), untreated gonadectomized (Gonad), gonadectomized mice treated with E2 (Gonad +E2), gonadectomized treated with DHT (Gonad + DHT) mice, and analyzed by bDNA signal amplification assay for Oct2 mRNA expression. The data are presented as mean RLU ± SEM (n=5). * and † represent a statistically significant difference ($p \leq 0.05$) between males and females, and control and treated animals, respectively.

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Table 1. Oligonucleotide probes generated for analysis of mouse Oct mRNAs expression by branched DNA signal amplification assay.

Target[a]	Function[b]	Sequence	Target	Function	Sequence
		Oct1 (NM_009202)			**Oct2 (NM_013667)**
673-691	CE	cacccagctgcctttgctgTTTTTctcttggaaagaaagt	1024-1045	CE	agagagactggcaccgattttcTTTTTctcttggaaagaaagt
817-833	CE	gctggagccagcgccagTTTTTctcttggaaagaaagt	1158-1180	CE	tggtagagaacagagctcgtgaaTTTTTctcttggaaagaaagt
850-872	CE	aatacagcaggaagaggaaggtgTTTTTctcttggaaagaaagt	1200-1216	CE	ttgtcccctgcgaggccTTTTTctcttggaaagaaagt
935-956	CE	gtgcaatttgctccattatcctTTTTTctcttggaaagaaagt	1408-1429	CE	gctatggtgatgcccattctacTTTTTctcttggaaagaaagt
1061-1079	CE	gggtgtgcttcctcaggctTTTTTctcttggaaagaaagt	998-1023	LE	catttttcttagcaatatgcttaatgTTTTTaggcataggacccgtgtct
692-714	LE	tctgtgatcaaggtgtagccagaTTTTTaggcataggacccgtgtct	1046-1065	LE	catctgccgtcaggctctgaTTTTTaggcataggacccgtgtct
715-735	LE	ctgtagccagagccgacaaacTTTTTaggcataggacccgtgtct	1066-1089	LE	ggttcaatttcatgccagtatcctTTTTTaggcataggacccgtgtct
736-758	LE	ggtacaagatggctgtcgttctcTTTTTaggcataggacccgtgtct	1134-1157	LE	ccaattgtacatcaagatcaacgtTTTTTaggcataggacccgtgtct
778-796	LE	cccagcaagccccactagcTTTTTaggcataggacccgtgtct	1181-1199	LE	catgtgcatgatgaggcccTTTTTaggcataggacccgtgtct
873-893	LE	gggattctgggacaaaccagtTTTTTaggcataggacccgtgtct	1244-1262	LE	agctgggaattccaccaggTTTTTaggcataggacccgtgtct
894-912	LE	tgagacaacagccaccgggTTTTTaggcataggacccgtgtct	1285-1303	LE	cggcgaccaatccggtctaTTTTTaggcataggacccgtgtct
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1024-1042	LE	tgcaaacgaaggactccgcTTTTTaggcataggacccgtgtct	1325-1343	LE	ggctgctcctgccaccataTTTTTaggcataggacccgtgtct
1080-1105	LE	agagaaccatagatacatcaggatgaTTTTTaggcataggacccgtgtct	1344-1365	LE	ggataaaaaccgaagctaggcaTTTTTaggcataggacccgtgtct
1106-1125	LE	ccctggtacagcacagcacaTTTTTaggcataggacccgtgtct	1366-1389	LE	ttttcagccactgtagatcatcagTTTTTaggcataggacccgtgtct
1126-1146	LE	gctcccacatgcatgatgaggTTTTTaggcataggacccgtgtct	1390-1407	LE	ccaagcacgccacggtaaTTTTTaggcataggacccgtgtct
1147-1166	LE	ggtagaggttggcccctgtgTTTTTaggcataggacccgtgtct	1430-1450	LE	accaggcagaccatttcgtagTTTTTaggcataggacccgtgtct
759-777	BL	cccactgtgaaggccacct	1090-1110	BL	tgaccaagtccaggaacgaag
797-816	BL	tctggaatggcataggccac	1111-1133	BL	atgtttccttatctgaggggttc
834-849	BL	ggcagggacaccgcca	1217-1243	BL	gcagagtagaagaaatccaagtagatg
913-934	BL	taccgcttgagtggttctcttc	1263-1284	BL	tggtgagaatgatgatgaaggc
957-977	BL	ggggcaccttcctgttcttct			
1001-1023	BL	ctctctgaggcatcttcctcaag			
1043-106	BL	gggggtgcggaacaggtc			
		Oct3 (NM_011395)			**Octn1 (NM_019687)**
2262-2284	CE	acctaatgggtgagagacttgctTTTTTctcttggaaagaaagt	993-1013	CE	ggatgatctgttcggcctctgTTTTTctcttggaaagaaagt
2306-2328	CE	tcctgagaactggagaactggttTTTTTctcttggaaagaaagt	1051-1074	CE	agctctagaggatcgaatatccctTTTTTctcttggaaagaaagt
2521-2540	CE	acctcagcacctgcaggagaTTTTTctcttggaaagaaagt	1098-1121	CE	ggtccaggattatgactttctgctTTTTTctcttggaaagaaagt
2713-2737	CE	ttttaaattctaagacctgggaaatTTTTTctcttggaaagaaagt	1319-1345	CE	gaatagcaccccagctataatatatctTTTTTctcttggaaagaaagt
2192-2215	LE	aacaggtccaagattatgtgttccTTTTTaggcataggacccgtgtct	970-992	LE	caaatctcctctgggatatcagcTTTTTaggcataggacccgtgtct
2216-2238	LE	gggtacctactgggtcttcatgaTTTTTaggcataggacccgtgtct	1014-1034	LE	tgttcatctttgcggctttctTTTTTaggcataggacccgtgtct
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2393-2416	LE	ggtgtgttgatcagatcagctgagTTTTTaggcataggacccgtgtct	1122-1143	LE	gcaatgttccgagtcctgaacaTTTTTaggcataggacccgtgtct
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2560-2582	LE	ttgtgttgcatttaaggagctgtTTTTTaggcataggacccgtgtct	1164-1185	LE	gaggttagcatccacagcatcaTTTTTaggcataggacccgtgtct
2583-2604	LE	aataccacagccaggtcctctgTTTTTaggcataggacccgtgtct	1210-1235	LE	catctccatgtaaattaggaacattgTTTTTaggcataggacccgtgtct
2605-2625	LE	gggactgcagcatgacttcacTTTTTaggcataggacccgtgtct	1346-1364	LE	gaagcacacctcctccccaTTTTTaggcataggacccgtgtct
2692-2712	LE	ccaaatccgtgctttgttttcTTTTTaggcataggacccgtgtct	1186-1209	BL	agagacagagcaaagtaacccact
2738-2766	LE	cacaaatatgacataattatccctttactTTTTTaggcataggacccgtgtct	1236-1259	BL	cagagaggaagcagttcaggtaga
2285-2305	BL	ggggccttttcatctctcctt	1260-1281	BL	taagctggaacttcaatcaggc
2329-2351	BL	gcttcctggatggtactgtcttg	1282-1302	BL	agtagcagccaggctgtgaag
2352-2372	BL	aggccttggtttgttcatcct	1303-1318	BL	ccgtggcagggttcgc

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2667-2691	BL	ttcctcaataaagagcctgtgatta

		Octn2 (NM_011396)			Octn3 (NM_019723)
592-615	CE	tctgagagctgtcctgaaatgaagTTTTTctcttggaaagaaagt	1480-1499	CE	ggtgattccaaacttccccaTTTTTctcttggaaagaaagt
770-792	CE	ttggaaagaatttctgttcccagTTTTTctcttggaaagaaagt	1646-1669	CE	cttcccatgaggatataaggtaggTTTTTctcttggaaagaaagt
793-815	CE	tggcgaatataattcgaattgacTTTTTctcttggaaagaaagt	1766-1789	CE	cttagggattgcctttgttttagtTTTTTctcttggaaagaaagt
986-1007	CE	tcacctctgcctctttaattcgTTTTTctcttggaaagaaagt	1833-1850	CE	ttcggaggttcgggaggtTTTTTctcttggaaagaaagt
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637-656	LE	tgcccatggtcaaaaacagcTTTTTaggcataggacccgtgtct	1500-1523	LE	cacatagaccatggagtaggcagaTTTTTaggcataggacccgtgtct
702-726	LE	aggacaaaaagcactgtaaacatctTTTTTaggcataggacccgtgtct	1546-1567	LE	acacccatgtttctgaccacagTTTTTaggcataggacccgtgtct
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1026-1047	LE	ggtgcaacaatcccattgatttTTTTTaggcataggacccgtgtct	1874-1896	LE	cattcagttcaatcagcttctggTTTTTaggcataggacccgtgtct
616-636	BL	acattcttgcgaccaaacctg	1897-1919	LE	tttctgacaacatgcaggttttcTTTTTaggcataggacccgtgtct
657-677	BL	ggaagctgaagccagtctgca	1524-1545	BL	tggggtacagctcagctgtgta
678-701	BL	cgaagttcacagagaagacctgca	1603-1628	BL	tagataaacaaagtagggagacagga
841-860	BL	gcagcaccatgaagccaaac	1695-1720	BL	ccagaactctctgggaagaataaagt
861-885	BL	tctctgatgaagtatgcaaacagtg	1721-1742	BL	aatggtttctgggagagaaacc
905-922	BL	ccctggcacagtgagcgc	1790-1808	BL	cttcggggacccttttcttg
944-963	BL	ggggactcagggatgaacca			

CE, capture extender; LE, label extender; BL, blocker.

[a] Target refers to the sequence of the mRNA transcript as enumerated in the GenBank file.

[b] Function refers to the utility of the oligonucleotide probe in the bDNA assay.

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Table 2. Summary of mouse tissues with predominant Octs mRNA expression.

Oct	Tissues of predominant expression
Oct1	Kidney, Liver
Oct2	Kidney
Oct3	Ovary, placenta
Octn1	All tissues except liver
Octn2	Kidney
Octn3	Testis

Figure 1

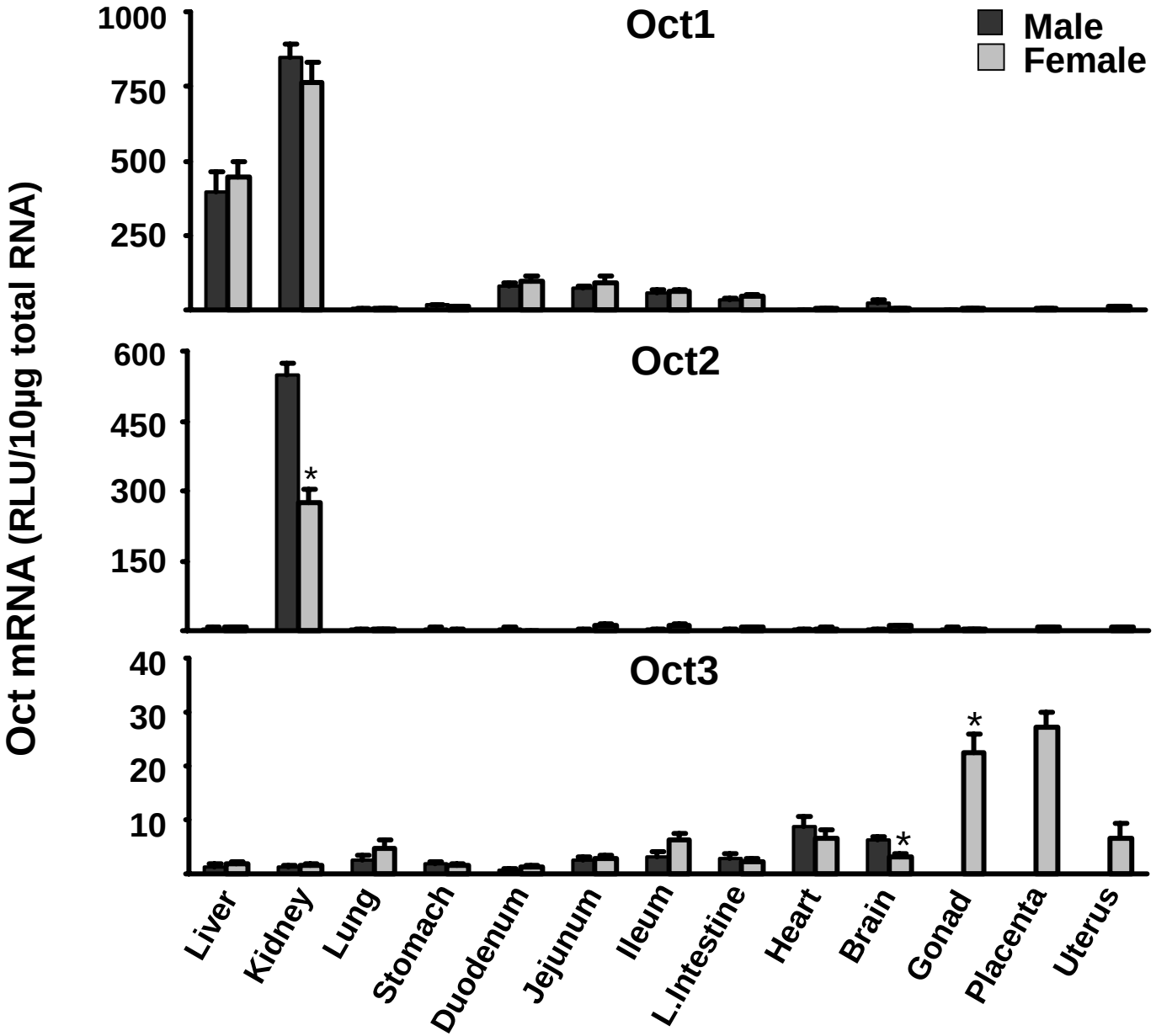

Figure 2

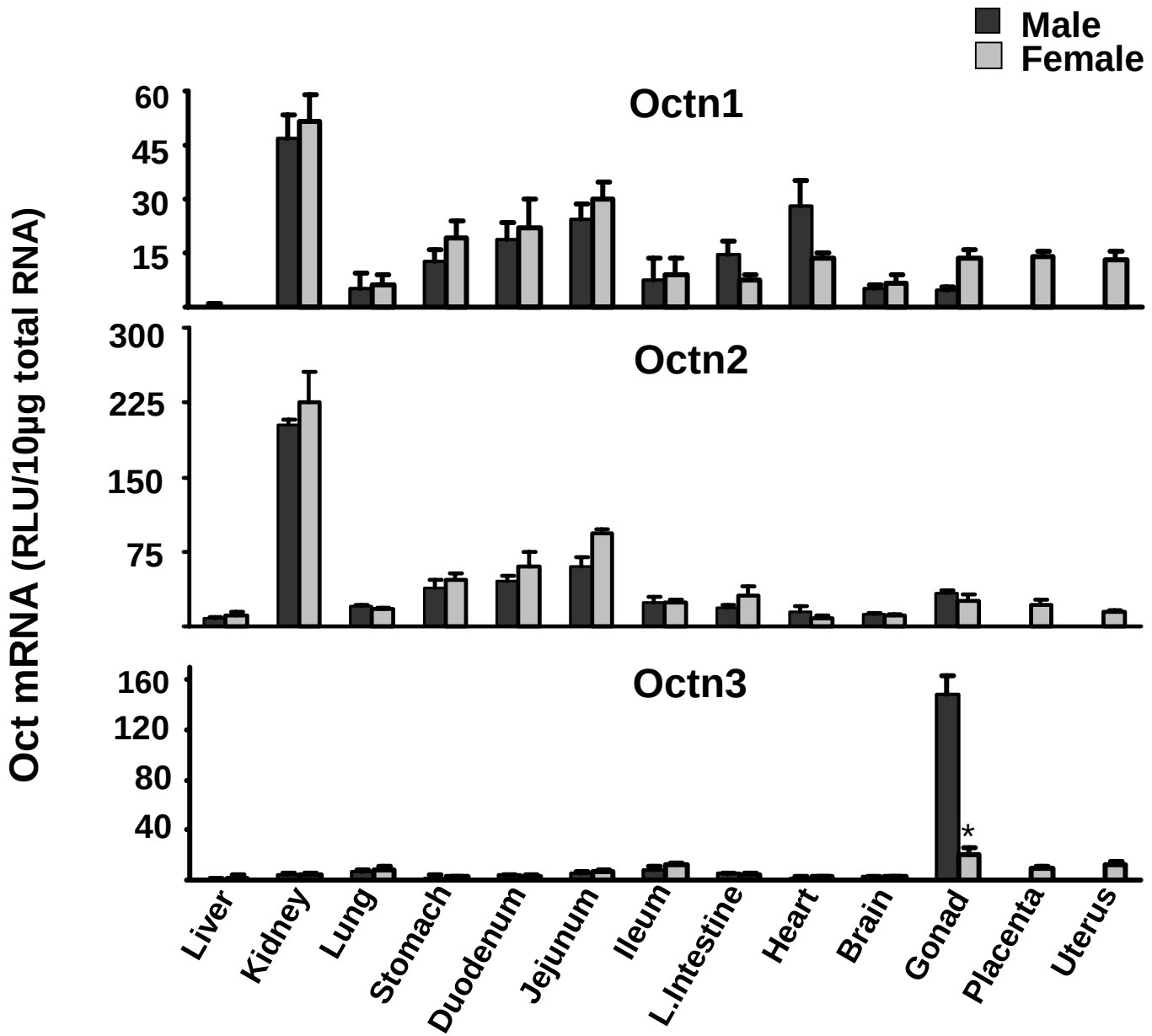

Figure 3

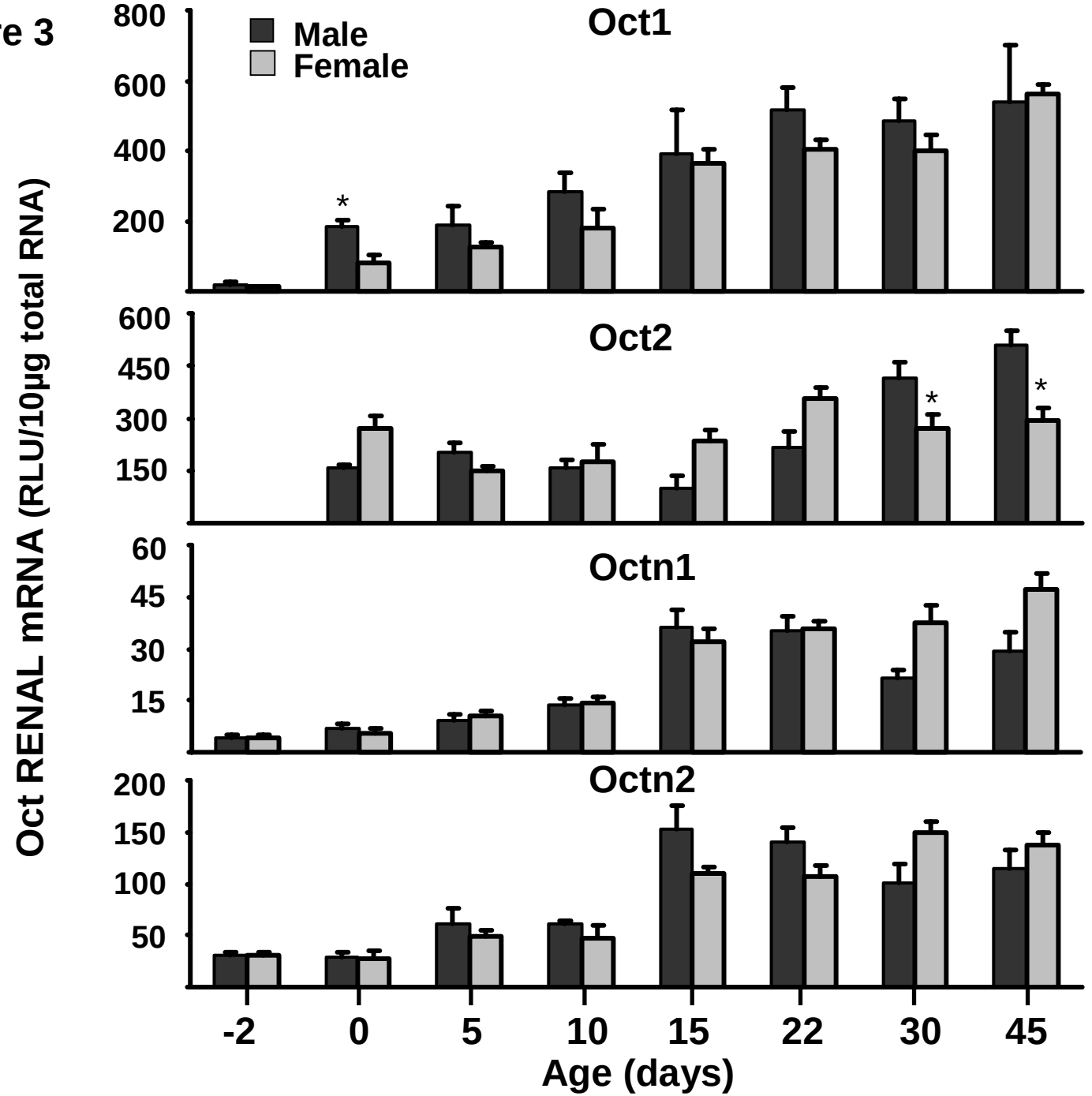

Figure 4

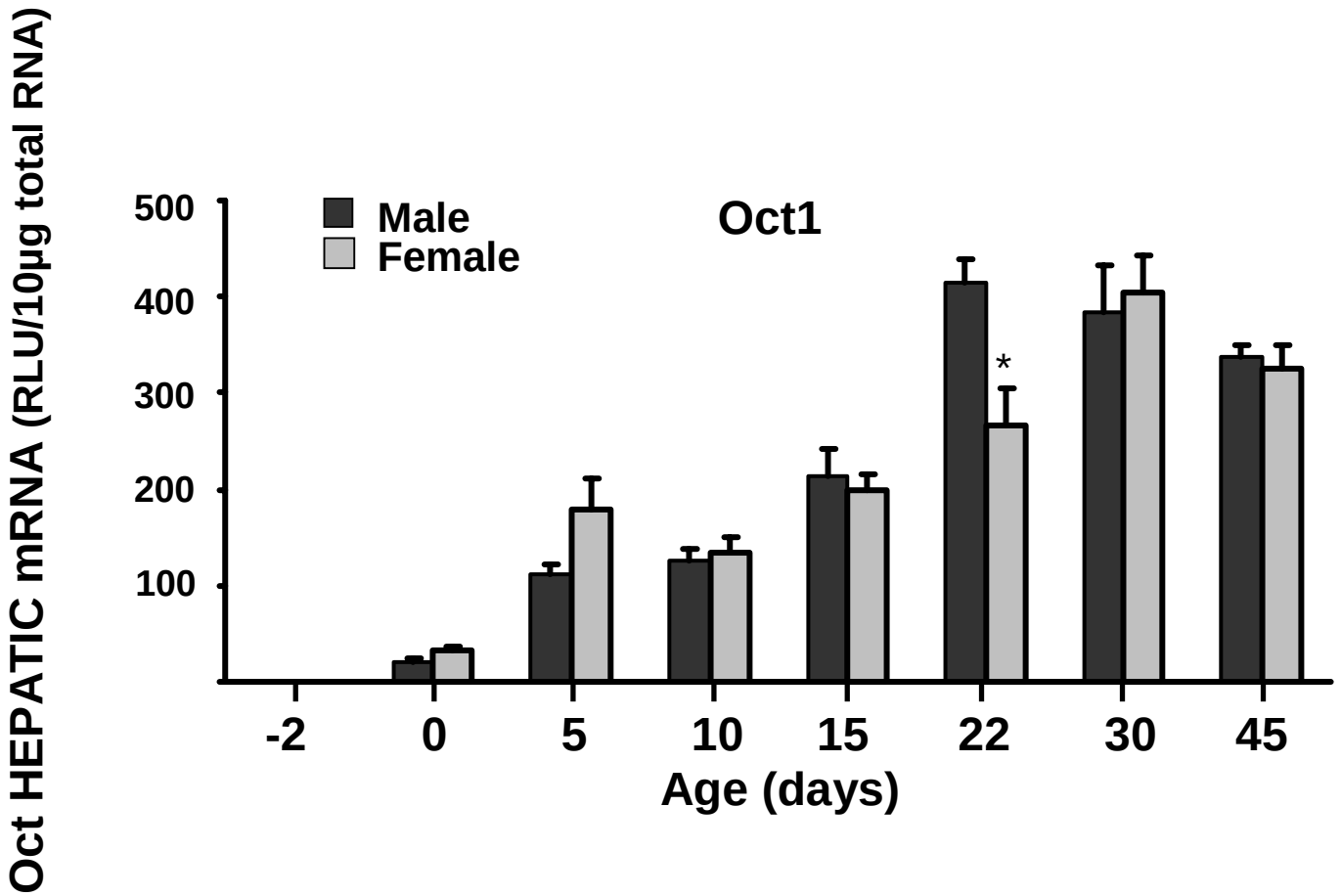

Figure 5