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Tissue distribution, ontogeny and induction of the transporters Multidrug and toxin extrusion (MATE) 1 and MATE2 mRNA expression levels in mice

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Abstract

Transporters are expressed in a wide variety of tissues where they perform the critical function of enabling anionic and cationic chemicals of exogenous and endogenous origin to cross otherwise impermeable cell membranes. The Multidrug and toxin extrusion (MATE) transporters mediate cellular efflux of a variety of organic cations, including many drugs. The purpose of the current study was to determine (1) constitutive expression levels of MATE mRNA in various tissues, (2) whether there are gender differences in the expression of MATEs, (3) the ontogenic expression pattern of MATE1 in kidney and (4) whether MATEs are pharmacologically-inducible in liver via activation of known transcription factors. In both male and female mice, MATE1 mRNA levels were highest in the kidney, where male expression was higher than female. MATE2 mRNA expression levels were the highest in the testis, where high expression was localized to Sertoli cells, a critical cell type of the blood testis barrier. In female mice, MATE2 mRNA levels were expressed most highly in the colon. The ontogenic pattern of expression of MATE1 mRNA in the kidneys of both males and females was gradual, with levels increasing steadily from prenatal day -2 to 45 days of age, and a gender difference appearing at day 30. Of the transcription factor activators examined (AhR, CAR, Nrf2, PPARα and PXR), none were capable of altering MATE1 or MATE2. The current findings support a potential role for MATE1 and MATE2 in a wide range of tissues and, notably, a unique role for MATE2 in the blood-testis barrier.

Keywords

Transporter; tissue distribution; testis; Multidrug and toxin extrusion; MATE1; MATE2

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Introduction

Transporter proteins fulfill many vital physiologic roles, as they are expressed in a variety of tissues and organs throughout the human body. For example, in intestinal and renal epithelial cells, the transporter known as the "sodium pump," or Na^+,K^+-ATPase helps maintain the osmotic balance of these cells, as well as provides a gradient for Na^+-dependent Na^+-glucose symport. Transporters are also responsible for driving bile salt-dependent and bile salt-independent bile flow, as well as the uptake and efflux of numerous structurally diverse endobiotics (e.g. bilirubin, bile acids, prostaglandins, sex steroids) and xenobiotics (e.g. aflatoxin B_1, digoxin, pravastatin, acetaminophen). Thus transporters help maintain electrolyte homeostasis, provide organ/tissue defense and likely contribute to the therapeutic efficacy of many drugs (Trauner and Boyer, 2003).

Among the endobiotics and xenobiotics that require transporter-mediated uptake and efflux, much is known regarding the handling of organic anions (OAs). Specifically, members of the Organic Anion Transporter (OAT) and Organic Anion-Transporting Polypeptide (OATP) families can transport OAs across cell membranes into tissues. Additionally, many OAT and OATP substrates as well as their metabolic products can undergo efflux out of cells for subsequent elimination from the body by members of the Multidrug Resistance-Associated Protein (MRP) and Multidrug Resistance (MDR) transporter families (Trauner and Boyer, 2003;Deeley et al., 2006;Anzai et al., 2006).

Similarly, members of the Organic Cation Transporter (OCT) family mediate the uptake of many Organic Cation (OC) endobiotics and xenobiotics across cell membranes into tissues. While relatively less is known regarding transporter-mediated OC efflux, evidence in the literature clearly supports the phenomenon of transporter-mediated OC efflux. Specifically, a family of proteins known as the Multidrug And Toxin Extrusion (MATE) transporters has been partially characterized in mice, rats and humans, and there appear to be two MATE genes in each of the species, MATE1 and MATE2 (Otsuka et al., 2005;Masuda et al., 2006;Terada et al., 2006).

In humans, MATE1 mRNA levels are highest in the liver, and are localized to the canalicular membrane of hepatocytes. MATE1 mRNA expression is also high in the kidneys, where it is localized to the apical membrane of the renal tubule. Similarly, MATE2 mRNA levels are by far at their highest in the kidneys, while relatively low in most other tissues. Example substrates for members of the MATE family include endogenous chemicals such as serotonin and testosterone and a number of drugs, including metformin and cisplatin (Otsuka et al., 2005;Terada et al., 2006;Masuda et al., 2006).

Previous work has demonstrated the tissue distribution of MATE family transporters in mice to be similar to that of humans (Masuda et al., 2006). However, a number of important issues regarding MATE expression in mice remain undefined. First, whether gender differences in mouse MATE1 and MATE2 exist is not known. The most obvious example where one could reasonably anticipate intergender disparities would be in the respective sex organs. Thus, gender differences in tissue distribution may reflect significant discrepancies in the physiologic role(s) of the transporter in question. Second, the ontogenesis of MATE1 and MATE2 mRNA expression has not been defined. This is a particularly important topic with respect to transporters because differences in expression during early development and adult maturation have been shown to result in differential susceptibility to injury (Guo et al., 2002;Johnson et al., 2002;Huang et al., 2000). Third, there is currently no information regarding the transcriptional regulation or pharmacologic inducibility of the MATE family of transporters. It is known that treatment with various microsomal enzyme inducers (MEIs) can result in induction of drug metabolizing enzymes (DMEs) and transporters, resulting in dramatic

alterations in the pharmacokinetics of prescription drugs (Klaassen, 1974;Guo et al., 2002). Pharmacologic induction of both DMEs and transporters has been demonstrated to occur following activation of various nuclear receptors, including Aryl hydrocarbon receptor (AhR), Constitutive androstane receptor (CAR), Pregnane x receptor (PXR), Peroxisome proliferator-activated receptor α (PPARα) and Nuclear factor erythroid 2 p45-related factor 2 (Nrf2) (Cheng et al., 2005;Maher et al., 2005). The purpose of the current study was to determine (1) the constitutive expression levels of MATE mRNA in various tissues, (2) whether there are gender differences in tissue distribution and expression levels of MATEs (3) the ontogenic expression pattern of MATE1 in kidney and (4) whether MATEs are pharmacologically-inducible in liver via activation of the nuclear receptor transcription factors AhR, CAR, PXR, PPARα and Nrf2.

Materials and Methods

Chemicals

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was a gift from Dr. Karl Rozman (University of Kansas Medical Center, Kansas City, KS). Oltipraz was a gift from Dr. Steven Safe (Texas A&M University, College Station, TX). Polychlorinated biphenyl 126 (PCB126) was obtained from AccuStandard (New Haven, CT). All other chemicals, unless otherwise indicated, were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Animals and Treatments

All male and female C57BL/6 mice (n = 10 per gender) were purchased from The Jackson Laboratory (Bar Harbor, ME), and tissues were collected at approximately 8 weeks of age. The following tissues were collected during necropsy: liver, kidney, lung, stomach, duodenum, jejunum, ileum, colon, heart, brain, testes, term placenta from pregnant dams (n = 5), and pooled ovaries (n = 5 samples; 1 sample = 10 ovary pairs from 10 individual mice). Ovaries were purchased from Charles River Breeding Laboratories (Portage, MI) from C57BL/6 mice undergoing ovariectomy for other experimental purposes. Tissues were snap-frozen in liquid nitrogen and stored at -80°C.

For the inducer study, groups of eight-week-old male C57BL/6 mice (n = 5) were administered one of the following chemicals once daily for 4 days: AhR ligands, TCDD (40 μg/kg i.p. in corn oil), β-naphthoflavone (βNF; 200 mg/kg i.p. in corn oil), and PCB126 (300 μg/kg p.o. in corn oil); CAR activators, TCPOBOP (3 mg/kg i.p. in corn oil), PB (100 mg/kg i.p. in saline), and diallyl sulfide (DAS; 200 mg/kg i.p. in corn oil); Nrf2 activators, butylated hydroxyanisole (BHA; 350 mg/kg i.p. in corn oil), oltipraz (OPZ; 150 mg/kg p.o. in corn oil) and ethoxyquin (EXQ; 250 mg/kg p.o. in corn oil); PPARα ligands, clofibrate (CLOF; 500 mg/kg i.p. in saline), ciprofibrate (CPFB; 40 mg/kg i.p. in saline), and diethylhexylphthalate (DEHP; 1000 mg/kg p.o. in corn oil); PXR ligands, PCN (200 mg/kg i.p. in corn oil), spironolactone (SPR; 200 mg/kg i.p. in corn oil), and dexamethasone (DEX; 75 mg/kg i.p. in corn oil). Four different vehicle control groups (corn oil by i.p., corn oil by p.o., saline by i.p., and saline by p.o. routes) were used. No statistical difference between these groups was observed; thus, these groups were averaged together as a single vehicle control group. All injections were administered in a volume of 10 mL/kg. Livers were removed on day 5, snap-frozen in liquid nitrogen, and stored at -80°C. With the exception of the Sertoli cell RNA, the RNA samples used in the current study were in fact derived from mice used in a larger series of studies that examined the inducibility of the Oatp family of drug transporters (Cheng et al., 2005). In Figure 1 of that particular manuscript, each drug was confirmed to be an inducer of its prototypical marker gene.

Sertoli Cell Isolation

Adult 8-9-week-old male C57BL/6 mice (Harlan, Dublin VA) were maintained on a 12-h alternating light/dark cycle and housed in a humidity- and temperature-controlled room on sani-chips with access to 4% mouse diet #7001 (Teklad) and water ad libitum. Animals were acclimated to housing conditions for at least 1 week before use. Animals were euthanized via carbon dioxide asphyxiation in accordance with the guidelines of University of Arizona's Institutional Animal Care and Use Committee in compliance with the National Institute of Health guidelines. Testes were removed and placed into 50 mL of Hanks Buffered Saline Solution (HBSS) (Mediatech, Herndon, VA; calcium/magnesium free, pH 7.4, adjusted with 7.5% sodium bicarbonate) and washed three times with sterile HBSS. Testes were de-capsulated and placed into sterile HBSS and minced into 2-mm cubes. Pieces were transferred into filter-sterilized trypsin solution containing 181 mg trypsin (Worthington Biochemical Corp., Lakewood, NJ), 210 units Dnase 1 (Stratagene), 48.1 mL HBSS, pH 7.2-7.4 and incubated for 30 min at 37°C. Samples were centrifuged at 200 x g, re-suspended and aspirated 10-15 times by pipet with 20 mL HBSS. After allowing tubules to re-settle, the supernatant was discarded. Tubule fragments were incubated for an additional 60 min at 37°C in a filter-sterilized collagenase solution [35 mg collagenase (Worthington), 252 units Dnase 1, 60 mg hyaluronidase (Sigma), and 35 mL HBSS, pH 7.2-7.4]. The mixture was centrifuged at 200 x g, supernatant removed and re-suspended/aspirated 10-15 times with 0.1% soybean inhibitor (Worthington). Hypotonic shock and filtering were carried out as described previously (Augustine et al., 2005). A 100 μL aliquot was used for purity analysis by identifying nuclear morphology. The remaining volume was snap frozen in liquid nitrogen for subsequent RNA isolation. Each isolate averaged greater than 60% Sertoli cells per animal.

Ontogeny

Mice (C57BL/6) were bred at the University of Kansas Medical Center laboratory animal facilities, and livers and kidneys were collected from male and female mice at -2, 0, 5, 10, 15, 23, 30, 35, 40, and 45 days of age (n = 5/gender/age).

RNA Extraction

Total RNA was isolated using the RNA Bee reagent (Tel-Test Inc., Friendswood, TX) according to the manufacturer's instructions. RNA concentrations were determined spectrophotometrically, and quality of RNA was determined by gel electrophoresis.

Development of Specific Oligonucleotide Probe Sets for bDNA Analysis

The mouse MATE1 and MATE2 gene sequences were accessed from GenBank, including accession numbers BC031436 and NM_001033542 for MATE1 and MATE2, respectively. ProbeDesigner Software Version 1.0 (Bayer Corp., Emeryville, CA) was used to analyze the target mRNA sequences. The probe design and target regions are shown in Table 1. The oligonucleotide probes were specific for only one mRNA transcript. All oligonucleotide probes were designed with a T_m of approximately 63°C, enabling optimal hybridization conditions. Each probe set was submitted to the National Center for Biotechnology Information for nucleotide comparison by the basic local alignment search tool (BLASTn) to ensure minimal cross-reactivity with other mouse genomic sequences and expressed sequence tags.

Branched DNA Assay

Oligonucleotide probes (either MATE1 mRNA-specific or MATE2-specific) were diluted in Tris-EDTA buffer, pH 8.0, according to instructions provided with the QuantiGene® bDNA Signal Amplification Kit (Panomics, Fremont, CA). Total RNA (1 μg/μl; 10 μl) was added to each well of a 96-well plate containing 50 μl of capture hybridization buffer and 50 μl of each diluted probe set. Total RNA was allowed to hybridize overnight at 53°C in a hybridization

oven. Subsequent hybridization steps were carried out according to the manufacturer's protocol, and luminescence was quantified with a Quantiplex 320 bDNA luminometer (Bayer Corporation, Diagnostics Division), interfaced with Quantiplex Data Management Software Version 5.02 for analysis of luminescence from 96-well plates.

Statistical Analysis

All values are expressed as relative light units (RLU) per 10 μg of total RNA (n = 5). Error bars represent standard errors of the mean (SEM). Intergender differences (Figures 1 and 2) were analyzed by a two-tailed Student's t test. Daggers represent statistical differences in mRNA levels between male and female mice ($p \leq 0.05$). Data of the microsomal enzyme inducer study (Figure 3) were analyzed by one-way analysis of variance, followed by Duncan's multiple range post hoc test.

Results

Tissue distribution of mouse MATE1 and MATE2 mRNA

MATE1 mRNA expression in both genders was quite low in most of the tissues examined. In both males and females, mRNA levels of MATE1 were highest in the kidney, followed by the liver and heart. MATE1 mRNA levels were significantly higher in female than male livers (by 37%). Conversely, MATE1 mRNA levels in the female kidney were significantly lower (by 31%) than in males. There were no other gender-dimorphic patterns of MATE1 mRNA expression in mice.

MATE2 mRNA was expressed at extremely highly levels in whole testis, including the Sertoli cells, whereas its expression was nominal in most other tissues. MATE2 mRNA levels in females were at their highest in the colon, followed by liver and lung. MATE2 mRNA levels in females were significantly lower than in males in several organs, i.e. 34% lower in the female colon, 68% lower in the heart and 97% lower in the female gonads (ovaries). MATE2 mRNA levels in males were by far at their highest in the gonads, followed by the colon and liver.

Ontogeny of MATE1 in mouse kidney

The mRNA expression levels of MATE1 in male and female mouse kidney, from prenatal day -2 to 45 days of age. In both males and females, the kidney mRNA levels of MATE1 increased steadily throughout the ontogenesis. At days 30 and 45, MATE1 mRNA levels were significantly lower in female kidney by 10% and 15%, respectively.

Effects of microsomal enzyme inducers on MATE1 and MATE2 mRNA levels in mouse liver

The mRNA levels of MATE1 and MATE2 in male mouse liver following treatment with various compounds that are established as microsomal enzyme inducers (MEIs) that activate distinct transcription factor pathways. Transcriptional induction of each of their respective phase I marker genes has been demonstrated previously to be mediated by the nuclear receptors AhR, CAR, PXR, PPARα and Nrf2 (Cheng et al., 2005). Notably, branched DNA analysis revealed that MATE1 and MATE2 are largely not inducible by prototypical microsomal enzyme inducers.

Discussion

The current study was carried out in order to more fully characterize the mRNA expression profile of MATE family transporters in mice. Although much new information regarding mRNA levels has been generated by the current study, it is important to note that mRNA levels are not necessarily reflective of protein expression or activity levels. These studies should

nonetheless generate greater interest in the functional contributions of MATEs in the tissues in which the mRNA was found at appreciable levels,

Before going further, it is also important to explain the current nomenclature conventions for the MATE family in humans and mice. Humans and mice each have the MATE1 gene, and MATE1 protein has observed to be functional in both species (~78% amino acid sequence identity) (Hiasa et al., 2006). Both humans and mice also have the MATE2 gene. While there is no functional MATE2 protein, MATE2-K, which is derived from a splice variant of the MATE2 gene, has been identified as a functional protein only in humans (Otsuka et al., 2005). On the other hand, in mice, there is a functional protein derived directly from the MATE2 gene (Hiasa et al., 2007). Given that hMATE2 and mMATE2 exhibit low mutual sequence identity (only 38%) a recent phylogenetic analysis was appropriate in placing them in entirely separate classes (Omote et al., 2006). It is therefore important to note that hMATE2 and mMATE2 should by no means be considered as orthologous isoforms.

Consistent with previously published reports, MATE1 mRNA expression is highest in the kidney, followed by brain, heart, liver and stomach (Otsuka et al., 2005;Hiasa et al., 2006). Additionally, MATE1 mRNA is expressed at dramatically lower levels in the colon, duodenum, ileum, jejunum, lungs, ovaries, placenta, Sertoli cells and whole testis. It is interesting to note that while MATE1 mRNA levels are low in whole testis, other studies have shown that protein expression is evident in Leydig cells of the testis (Hiasa et al., 2006).

Also in line with previous reports, MATE2 mRNA levels are at their highest in testis (Otsuka et al., 2005). However, it was important to determine in which sub-population of cells MATE2 is expressed because the testis cell population is diverse in type and function. For example, spermatogenic cells include those that will develop from immature spermatogonia into mature spermatozoa for reproduction. Leydig cells, which are the resident endocrine cell of the testis, are localized to the spaces between adjacent seminiferous tubules, from which they secrete the important male sex hormone testosterone. Sertoli cells, which are embedded among the spermatogenic cells in the seminiferous tubules, support and protect developing spermatogenic cells. In order for a chemical, whether endogenous or exogenous, to reach developing sperm cells, it must first pass through the Sertoli cells, which form the functional blood-testis barrier.

The relatively high expression of MATE2 mRNA in the Sertoli cell population would support a hypothesis that MATE2 is an important mediator of the transepithelial transport of cationic drugs and other exogenous and endogenous cationic compounds across the blood-testis barrier. For example, high concentrations of some nucleoside analog drugs have been detected in the semen. It is likely that these drugs were taken up into Sertoli cells by Equilibrative Nucleoside Transporters, and then putatively effluxed into the adluminal compartment by MATE2 (Cruciani et al., 2006).

The second objective of this study was to examine the possibility of gender differences in MATE mRNA expression. MATE1 showed a moderate gender divergence in the liver, where it was 37% higher in the female, and in the kidney, where it was 31% lower in the female. This was an interesting finding given that no MATE1 protein expression in rats is not gender divergent (Nishihara et al., 2007). For MATE2, the mRNA levels were significantly lower in female colon (34%), heart (69%) and the gonads (97%). The observed intergender differences in mRNA expression levels for the mouse MATE family transporters were overall rather modest. With the exception of the gonads, it is likely that MATE2 has equivalent physiologic roles in male and female mice.

Susceptibility of neonates to potential adverse effects induced by various chemicals has long been of concern to clinicians. The relevance of transporters during development is highlighted

by the example of Oatp2 and cardiac glycosides. Neonatal rats are sensitive to cardiac glycoside toxicity because expression of the uptake transporter Oatp2 is low in liver, thereby prolonging systemic exposure to the drug and increasing the susceptibility to toxicity (Klaassen, 1973). Mrp2 provides another example, i.e. neonatal jaundice may be due partly to weak expression of this canalicular transporter in the liver (Johnson et al., 2002;Huang et al., 2000). In the current study, ontogenic expression of MATE1 was examined in kidney, where its adult expression is by far the highest and where there is considerable evidence that MATE1 plays a significant role in the renal excretion of organic cations (Nishihara et al., 2007). MATE1 mRNA levels were much lower in kidneys during the early stages of development, most notably at prenatal day 2, when expression was 12% and 14% of day 45 in males and females, respectively. It was not until day 15 that MATE1 mRNA expression finally reached at least half of that in 45 day-old male and female kidneys. This dramatic difference in expression between the neonate and the adult mouse may reflect differences in susceptibility to injury following exposure to chemicals that are normally eliminated via tubular secretion by kidney MATE1. The fact that the ontogenic pattern of MATE1 expression between the male and female was similar (levels continued to increase steadily through day 45) indicates that the susceptibility to injury and functional role(s) of MATE1 in the kidney may be similar among the genders.

The final component of the current study was to examine the inducibility of transporters of the mouse MATE family. Treatment with a variety of microsomal enzyme inducers (MEIs) largely failed to produce any significant changes, i.e. neither up-regulation nor suppression, in mRNA levels of MATE1 or MATE2 in the liver. Ciprofibrate (CPFB) produced a 35% increase in MATE1, but this change was not statistically significant. Conversely, although not statistically significant, each of the PPARα agonists appeared to suppress mRNA expression levels of MATE2. This was an interesting phenomenon because down-regulation of efflux transporters following MEI treatment is not a typical response. It is not clear why the liver would respond to an incoming xenobiotic by suppressing transcription of a xenobiotic transporter that could potentially protect the same organ. The general lack of inducibility of the MATEs was nonetheless a surprising result, given the large number of endobiotic and xenobiotic transporters that have been demonstrated to be inducible or repressible following activation of those nuclear receptors tested here (AhR, CAR, Nrf2, PPARα and PXR) (Cherrington et al., 2002;Cheng et al., 2005;Maher et al., 2005). Several members of the ATP-Binding Cassette family C (ABCC) or Multidrug resistance-associated protein (Mrp) family, including Mrp2, 3, 4, 5 and 6, are inducible following pharmacologic activation of either CAR or Nrf2 (Maher et al., 2005). In addition to pharmacologic induction, many of these same Mrp transporters have been demonstrated to undergo differential regulation in a variety of tissues during models of disease, including experimental obstructive cholestasis (Slitt et al., 2007). It will thus be interesting to see whether any of the MATEs undergo differential regulation in response to various environmental stimuli or disease models. Such studies may provide additional insight into the physiologic role(s) of MATEs.

The MATE family of transporters has been demonstrated in both prokaryotes and eukaryotes to be responsible for the extrusion of OCs from cells (Omote et al., 2006;Zhang et al., 2007). It is also generally accepted that the mechanism of OC secretion involves coupled OC/H^+ exchange, although the overall contribution of MATEs to total OC flux is not known (Terada et al., 2006). Regardless, the identification of those cationic xenobiotics and endobiotics that are MATE substrates will be fundamental to understanding the pharmacologic and toxicologic significance of this recently identified family of transporters. One clinically relevant example of the significance of transporters in OC excretion involves the drugs cimetidine and procainamide. Co-administration of these cationic drugs, both of which have been recently revealed as substrates of human MATE1, has been observed to reduce the renal clearance of cimetidine by 44% in human patients (Otsuka et al., 2005;Somogyi et al., 1983). Clearly, it will be important to know which compounds are substrates or inhibitors of MATEs and thus

capable of blocking the excretion of metabolic products and/or xenobiotics. This should also enable scientists in the field of preclinical drug development to better understand the pathways and routes of elimination for various candidate cationic drugs and xenobiotics.

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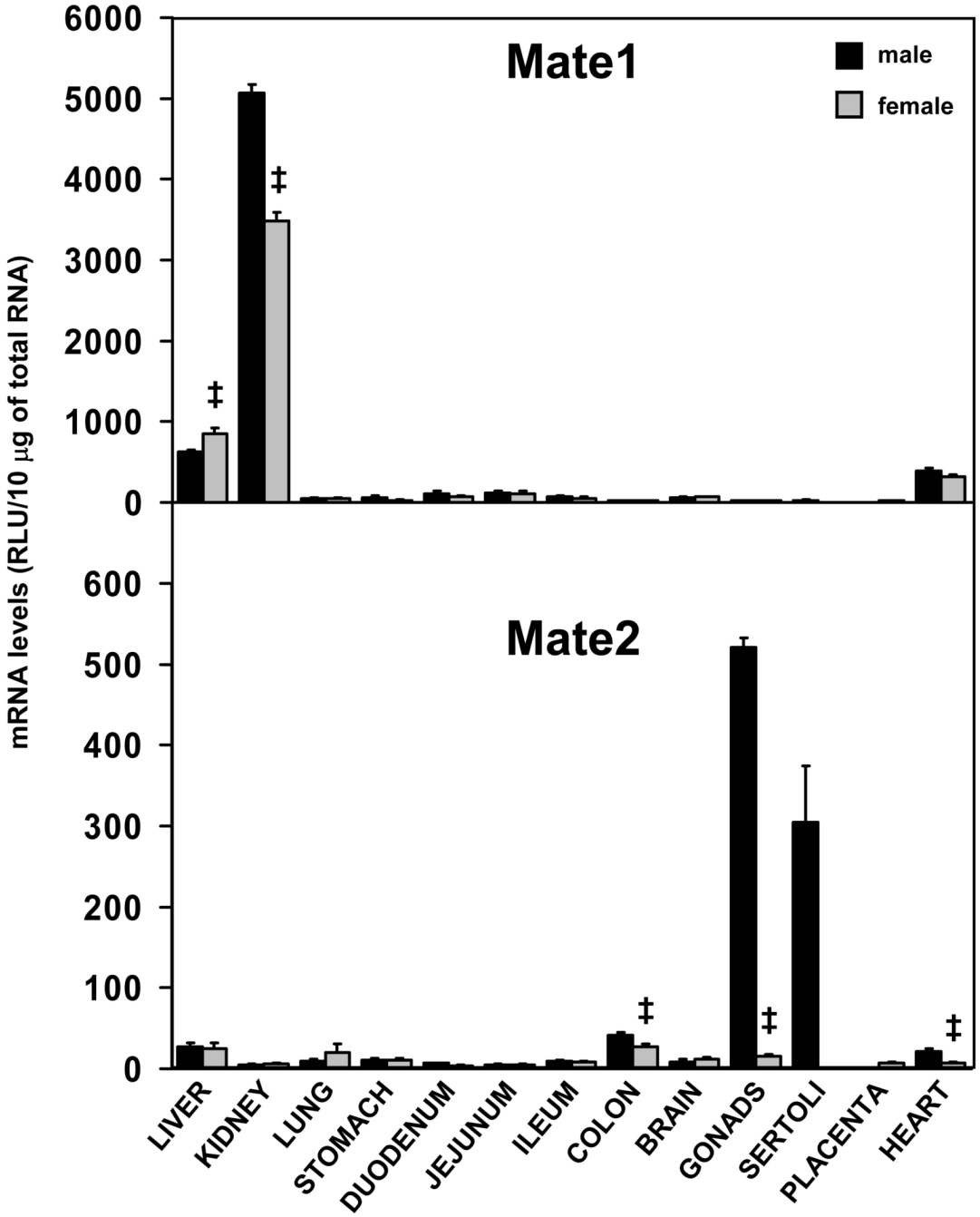

Figure 1.

Tissue distribution of MATE1 and MATE2 mRNA. Total RNA from both male and female C57BL/6 mouse tissues (n = 10/gender) was analyzed by the bDNA assay. Data are presented as the mean ± Standard Error of the Mean (SEM). Daggers indicate statistically significant differences between male and female mice ($p \leq 0.05$).

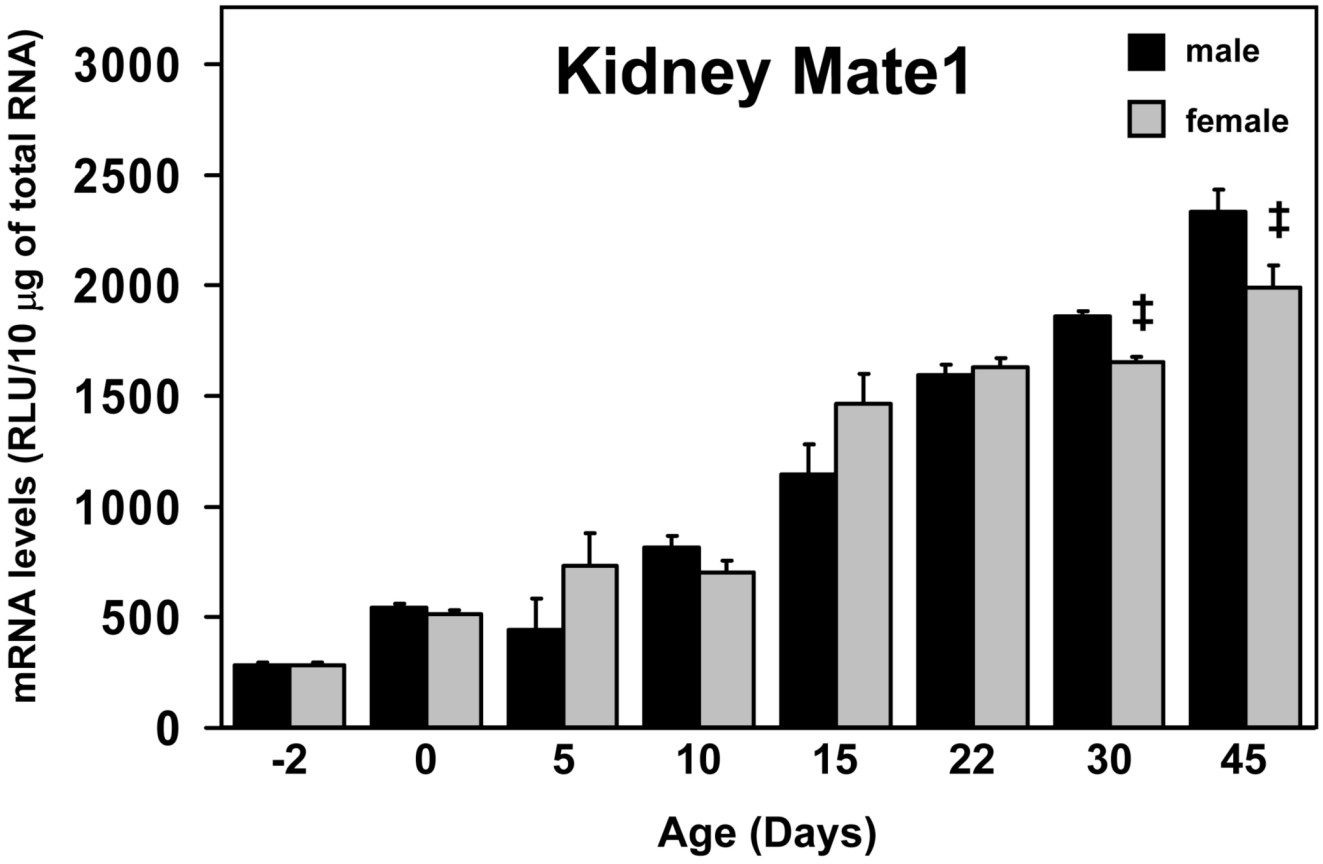

Figure 2.

Ontogenic expression of mouse MATE1 and MATE2 mRNA in mouse kidney. Total RNA from C57BL/6 mice at each age (n = 5/gender) was analyzed by the bDNA assay. Data are presented as mean ± SEM. Daggers indicate statistically significant differences between male and female mice ($p \leq 0.05$).

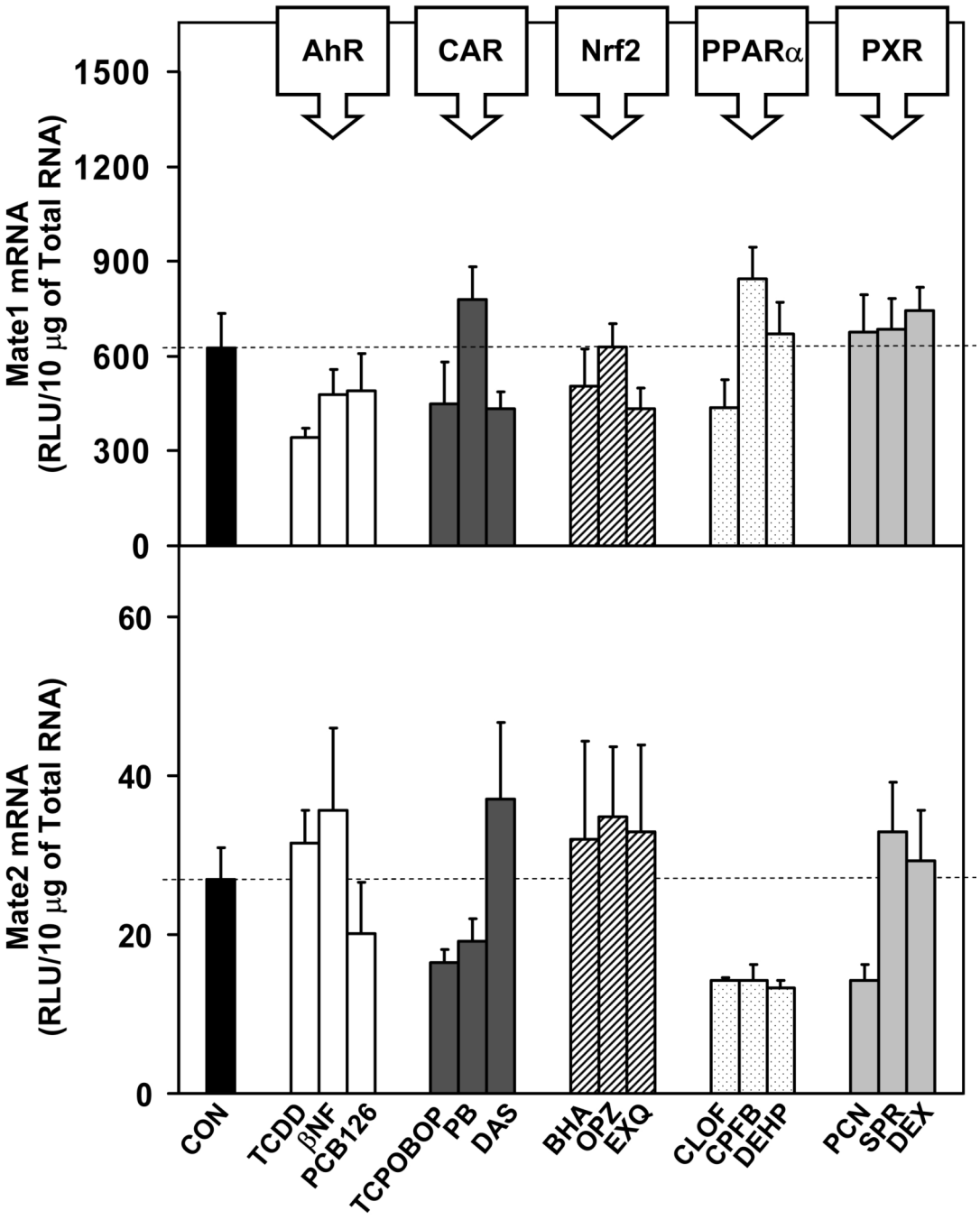

Figure 3.

Expression of MATE1 and MATE2 mRNA in liver after treatment with prototypical drug-metabolizing enzyme inducers. Total RNA from liver of chemically-treated male C57BL/6 mice (n = 5/treatment) was analyzed by the bDNA assay. All data were expressed as mean ± SEM for five animals in each group, except for control groups, which were combined from the four individual control groups after it was determined that they were not statistically different.

Table 1

Oligonucleotide probes generated for analysis of mouse Mate expression by bDNA signal amplification

Gene (accession no.)	Function	Sequence
Mate1 (BC031436)	CE	ggttcccagtgcccctcagTTTTTctcttggaaagaaagt
	CE	gcgcgtttccaattgagcTTTTTctcttggaaagaaagt
	CE	gcgcgtttccaattgagcTTTTTctcttggaaagaaagt
	CE	tgctggattgatggcattccTTTTTctcttggaaagaaagt
	LE	gataccaccacaggtacaagcaaTTTTTaggcataggacccgtgtct
	LE	taacgatggctccaaccttctTTTTTaggcataggacccgtgtct
	LE	atgacataatacccgatggcatTTTTTaggcataggacccgtgtct
	LE	cagcaaacatcagcgcgatTTTTTaggcataggacccgtgtct
	LE	gccgatcactcccagcttagTTTTTaggcataggacccgtgtct
	LE	agatgatgatgcctgaccacagTTTTTaggcataggacccgtgtct
	LE	gcatgtacttgagcctgttgacagTTTTTaggcataggacccgtgtct
	LE	ggccacgtttaccttcaaattgTTTTTaggcataggacccgtgtct
	LE	acacactgggtgagcaggctTTTTTaggcataggacccgtgtct
	LE	ttttttttcaagatccgtcatcaTTTTTaggcataggacccgtgtct
	LE	gctggtccaactgagtctcgtctTTTTTaggcataggacccgtgtct
	LE	aagcttgttgctggttcatcgTTTTTaggcataggacccgtgtct
	LE	tgtttcccagacagtttattgctTTTTTaggcataggacccgtgtct
	LE	agtcctctccgtaaggccaacTTTTTaggcataggacccgtgtct
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	LE	agccctgatgtttcaatgtggTTTTTaggcataggacccgtgtct
	BL	cccgatggggaggcca
	BL	cacgtggtctgacaggtagtgc
	BL	cgagcaataaaagccaggaag
	BL	cgtgagagacagcggaattcag
	BL	gtcctttggacggataggca