Postnatal Ontogeny and Hormonal Regulation of Sulfotransferase SULT1B1 in Male and Female Rats

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ABSTRACT

The ontogenic and hormonal regulation of a sulfotransferase, SULT1B1, was examined. Hepatic RNA was isolated from rats of various ages from 1 to 90 days. The mRNA for SULT1B1 is low for both sexes until a dramatic increase (~6-fold) occurs between 15 and 30 days of age in male rats. SULT1B1 expression then decreases to half of the maximal level by 90 days of age. The increase in SULT1B1 mRNA in female rats is less dramatic and occurs between 30 and 45 days of age. SULT1B1 mRNA expression plateaus from 45 to 90 days in female rats. Expression of SULT1B1 mRNA is comparable in adult male and female rats. RNA was isolated from hypophysectomized (HX) animals and HX animals treated with growth hormone [by either male (injection) or female (infusion) pattern], estradiol, progesterone, or testosterone. HX and HX plus growth hormone, or HX plus steroid replacement, did not alter SULT1B1 mRNA expression. Pituitary-intact rats were treated with steroidal compounds dexamethasone (DEX) and pregnenolone-16α-carbonitride (PCN). Both DEX and PCN increased expression of SULT1B1 mRNA in male rats (4- and 3-fold, respectively). However, in female rats, only PCN induced SULT1B1 mRNA (2-fold), whereas DEX did not induce SULT1B1 in female rats. Analysis of SULT1B1 protein expression indicated that only when SULT1B1 mRNA was markedly increased, that is in DEX-treated male rats, was SULT1B1 protein increased. Thus, although adult male and female rats have similar SULT1B1 mRNA expressions, the patterns develop ontogenically differently. SULT1B1 is not regulated by pituitary hormones and DEX induces SULT1B1 protein in male rats.

Sulfotransferases (SULTs) are a family of phase II drug-metabolizing enzymes involved in xenobiotic detoxication (e.g., acetaminophen), bioactivation of drugs like minoxidil (Falany and Kerl, 1990), and activation of certain carcinogens such as N-hydroxy-2-ace
taminofluorene (DeBaun et al., 1970), safrole (Borchert et al., 1973), and hydroxymethylenebenzanthracene (Surh et al., 1991). Additionally, SULTs metabolize endogenous compounds, such as sex steroids and glucocorticoid hormones, as well as some neurotransmitters (Brooks et al., 1978; Penke et al., 1985; Rajkowski et al., 1997). SULTs use the activated sulfate donor 3′-phosphoadenosine-5′-phosphosulfate to catalyze the transfer of a sulfuryl functional group from the activated sulfate donor to substrates (Klaassen and Boles, 1997), which leads to an enhanced water solubility for these compounds to be excreted. In contrast, the addition of a sulfuryl moiety can create highly reactive, electrophilic compounds that form covalent adducts with macromolecules such as nucleic acids (Okuda et al., 1989).

SULT nomenclature has previously been based on substrates. The mammalian SULTs are divided into two major families: SULT1, “phenol” SULTs, and SULT2, “hydroxy-steroid” SULTs (Fujita et al., 1997). However, a new SULT nomenclature system modeled after the cytochrome P-450 nomenclature is being reviewed. The new nomenclature will identify each SULT with respect to its unique cDNA sequence to remove the ambiguity that exists with substrate-based nomenclature.

A new member of the phenol SULT family of enzymes has recently been identified. This isoform is designated as SULT1B1 and is extremely similar to another enzyme, dopa/tyrosine SULT (Sakakibara et al., 1995; Fujita et al., 1997). Only 1 amino acid difference results from 12 nucleotide differences between the two cDNAs, and the enzymes have very similar substrate kinetics (Sakakibara et al., 1995; Fujita et al., 1997). Thus, the physiological significance of the apparent sequence differences between SULT1B1 and dopa/tyrosine SULT has not been established. However, it is known that SULT1B1 is expressed at similar levels in male and female rats.

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ABBREVIATIONS: SULT, sulfotransferase; SSC, standard sodium citrate; GH, growth hormone; HX, hypophysectomy; ELISA, enzyme-linked immunosorbent assay; PBS-T, PBS containing 0.05% Tween-20; EB, estradiol benzoate; TP, testosterone propionate; PR, progesterone; DEX, dexamethasone; PCN, pregnenolone-16α-carbonitride; ABTS, 2,2′-azino-di[3-ethoxybenzyl thiozoline sulfonate].
female rats and that this isozyme is present in both liver and kidney (Araki et al., 1997). The mRNA for SULT1B1 has also been detected in the liver, intestine, and kidney of male and female rats (Dunn and Klaassen, 1998).

The hormonal regulation of SULT1B1 expression is still unknown. Other SULT mRNAs are responsive to growth hormone (GH) secretion patterns and certain steroidal compounds, including dexamethasone (DEX) and pregnenolone-16α-carbonitrile (PCN) (Liu and Klaassen, 1996a,b,c) and progesterone (PR) (Meyers et al., 1983). Certain SULT enzymes are also responsive to xenobiotics, including 3-methylcholanthrene (Runge-Morris and Wilusz, 1994).

Specific oligonucleotides that can distinguish between SULTs at the level of their respective mRNAs are now available. Our previous work, using specific oligonucleotides, has delineated hormonal responsiveness of six major SULTs in male and female rats (Liu and Klaassen 1996a,b,c). In addition, oligonucleotides have been used as probes to assess the tissue-specific distribution of SULT mRNAs in male and female rats (Dunn and Klaassen, 1998). The purposes of the present study were to analyze the ontogenic expression and to define the hormonal responsiveness of SULT1B1 mRNA.

Materials and Methods

Reagents and Buffers

All reagents were of molecular biology grade (Sigma Chemical Co., St. Louis, MO) and were used as described previously (Liu and Klaassen, 1996a,b,c). 3-(N-Morpholino)propanesulfonic acid buffer consisted of 0.2 M 3-(N-morpholino)propanesulfonic acid, 0.05 M sodium acetate, and 0.01 M EDTA, pH 7.2, which was diluted 10-fold with diethylpyrocarbonate-treated ddH2O before use. Prehybridization and hybridization solutions were obtained from Sigma Chemical Co. Zetaprobe GT blotting membranes were from Bio-Rad (Hercules, CA). Ultrapure agarose was purchased from GIBCO BRL (Gaithersburg, MD). Rat GH (1.8 IU/mg AFP-87401) was a generous gift from the University of Kansas Medical Center. The oligonucleotide probe was based on published cDNA sequences and synthesized by Biotechnology Support Facility at the University of Kansas Medical Center. The oligonucleotide was assessed for uniqueness by BLAST searches of the GenBank nucleotide sequence databank. The oligonucleotide was designed to be complementary to a certain divergent sequence of the respective cDNA. The SULT1B1-specific oligonucleotide was complementary to nucleotides 814–834 of the cDNA sequence reported by Sakakibara et al. (1995) and complementary to nucleotides 882–903 of the sequence reported by Fujita et al. (1997). The SULT1B1 oligonucleotide detects both of the highly similar SULT isoforms, SULT1B1 and dopa-tyrosine SULT.

The oligonucleotide was labeled with [α-32P]dATP (6000 Ci/mm mol) (Amersham, Arlington Heights, IL) by tailing with terminal deoxynucleotidyl transferase (Boehringer-Mannheim, Indianapolis, IN). Oligonucleotide labeling reaction was terminated by the addition of 5 μl (10% v/v) of 0.5 M EDTA. Labeled oligonucleotide was chromatographically purified using G-25 (fine) Sephadex (Pharmacia, Piscataway, NJ) spin columns (Boehringer-Mannheim).

Northern Blot Analysis

RNA was transferred onto nylon membranes by capillary action in 10× standard sodium citrate (SSC) (1× SSC = 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0). Membranes were dried for 1 h at 70°C and then cross-linked under UV light, followed by prehybridization (4 h) and hybridization overnight (~18 h) with a [32P]-labeled oligonucleotide probe specific for SULT1B1 (5′-TCC AGA CAA TTT CTT CTP-3′). Hybridization was performed at 46°C in 20% formamide. The membranes were washed twice in 2× SSC in 2% SDS for 20 min at 46°C and then washed once in 1× SSC in 2% SDS at 46°C, followed by a final wash in 1× SSC in 2% SDS at 50°C. Hybridization signals were detected and quantified after exposure to phosphor screens and analysis by phosphorautoradiography using Image Quant software (Molecular Dynamics, Sunnyvale, CA).

Peptide-Specific Antibodies to SULT1B1

Selection of SULT1B1 Peptide. To produce monospecific antibodies directed against SULT1B1, the SULT1B1 amino acid se-
quence was selected from the previously published sequence of the “dopa/tyrosine” SULT (Sakakibara et al., 1995). A 12-amino-acid peptide (GTAEDVFRKDLK) corresponding with the N-terminal region of SULT1B1 (amino acids 2–13) was identified as a region of SULT1B1 nonhomologous to other SULT family members by BLASTp analysis. This peptide appeared sufficient for use in generating polyclonal antibodies by computational Kyte-Doolittle analysis (−0.75 mean hydrophobicity score) (Kyte and Doolittle, 1982). The peptide was synthesized and HPLC-purified by the University of Kansas Medical Center Biotechnology Support Facility.

**Generation of SULT1B1 Peptide-Carrier Protein Conjugates.** The SULT1B1 peptide contains an artificial N-terminal cysteine residue that was added (GTAEDVFRKDLK; MW = 1427 g/mol; 71% recovery) to facilitate cross-linkage of the peptide to the carrier protein. To generate carrier protein-peptide hapten conjugates, the N-C-SULT1B1 peptide (1.2 mg; 0.6 μmol) was dissolved in 100 μl of PBS, pH 7.4, mixed with maleimide-activated keyhole limpet hemocyanin (2 mg; 0.6 μmol of maleimide; Imject Activated SuperCarrier System; Pierce, Rockford, IL) and incubated for 2 h at room temperature (Sharp et al., 1995). After the incubation, the sample was filtered through a Centricon concentrator (30,000-Da cutoff; Amicon, Inc., Beverly, MA), the filtrate was retained for analysis of residual peptide-derived cysteine content, and the carrier protein-hapten conjugate was washed (three times) with PBS. Washed and concentrated samples (100 μl/1 mg conjugate) were diluted in PBS (0.4 ml). Cysteine content in the initial filtrate was assayed using Ellman’s reagent (Dimonte et al., 1984), and the total incorporation of SULT1B1 peptide into KLH was determined by back calculating from residual cysteine in the filtrate. At least 90% of SULT1B1 peptide was incorporated into the carrier protein, yielding the SULT1B1-KLH conjugate. This conjugate was used as the immunogen for antibody production in rabbits.

**Immunization of Rabbits and Generation of Antiserum.** For production of anti-SULT1B1 sera, carrier-protein-SULT1B1 conjugates (1.0 mg/0.5 ml PBS) were suspended in Freund’s complete adjuvant (0.5 ml) for the priming injection and Freund’s incomplete adjuvant for subsequent booster injections. To initiate an immune response, New Zealand White rabbits (two rabbits per antigen) were given primary injections of the antigen in eight sites (s.c.; 100 μg antigen/site) as described by Harlow and Lane (1988). After the initial priming injection, animals were given two booster injections at 28-day intervals and test bleed 10 to 14 days after each booster injection. Blood samples (5–10 ml) were obtained from the rabbit’s ear vein. All animals were given two booster injections, and after assessment of SULT1B1 peptide hapten-specific antibody titers by enzyme-linked immunosorbent assay (ELISA), the animals were exsanguinated. Whole blood was immediately refrigerated and allowed to coagulate, followed by serum separation via centrifugation and storage at −30°C.

**Verification of Antibody Titers to SULT Peptides with Antibody-Capture ELISAs.** To validate the generation of antibodies to the various SULT peptides, the SULT1B1 peptide was used in ELISA and in initial Western analyses. Because these peptides do not bind to microtiter plates efficiently or separate electrophoretically on standard polyacrylamide gel systems, each peptide was cross-linked to maleimide-activated BSA (mBSA; Pierce). Briefly, mBSA (2.0 mg; 0.51 nmol maleimide; 17 mol maleimide/mol BSA mg/ml) was incubated with the SULT1B1 peptide (0.7 mg; 0.51 nmol peptide) at 4°C overnight. Excess reagents were removed by filtration as above. These peptide-BSA conjugates were used in ELISA analyses (1 μg SULT1B1-BSA conjugate/well).

The SULT1B1-BSA conjugates were applied to Immunolone 4 96-well Microtiter Immunoassay Plates (Dynatech Laboratories, Inc., Chantilly, VA) at a concentration of 0.5 μg/50 μl PBS/well, and the peptide-BSA conjugate was allowed to bind to the well overnight at 4°C. Subsequently, the conjugate solution was removed, wells were rinsed three times with PBS containing 0.05% Tween-20 (PBS-T), and protein-binding sites in each microtiter well were blocked with a solution of 1% BSA (w/v) in PBS-T (300 μl/well) overnight at 4°C. Blocking solution was removed, the wells were rinsed (three times) with PBS-T, and serial dilutions of each antibody (1:25 to 1:25,000 in PBS-T) were aliquoted into appropriate wells (50 μl/well) and allowed to incubate for 2 h at room temperature. Plates were rinsed (three times) with PBS-T. Secondary goat anti-rabbit IgG conjugated to horseradish peroxidase were added to the wells and incubated for 30 min (1:5000 in PBS-BSA). Plates were rinsed (three times) with PBS-T, and horseradish peroxidase ABTS (2,2′-azino-di[3-ethylbenzyl thiazoline sulphonate]/Mellwain’s substrate solution was added to each well. ABTS/Mellwain’s solution consists of 1.0 ml of 10 mg/ml ABTS in water, 8.95 ml of Mellwain’s solution (13.3 ml of 0.1 M citric acid and 11.7 ml of 0.2 M sodium phosphate, pH 4.6), and 50 μl of 1% hydrogen peroxide. The substrate solution was added to each well (50 μl/well). The reaction was allowed to develop for 15 min at 37°C. Antibody-dependent absorbance was measured spectrophotometrically at 405 nm with a 96-well microplate reader (Molecular Devices Corp.), and the data were analyzed using Softmax Version 2.32 software (Molecular Devices).

**Western Blot Analysis**

Samples of liver tissue from the DEX- and PCN-treated rats were homogenized in buffer (5× volume of 20 mM Tris, 6 mM 2-mercaptoethanol, pH 7.5) using a Teflon pestle and a 15-ml glass homogenizing vessel (Wheaton, Millville, NJ). Each homogenate was centrifuged for 1 h at 100,000g to obtain the cytosolic fraction. After centrifugation, the protein concentration of each cytosol was assayed by the bicinchoninic acid procedure (Smith et al., 1985) using a kit supplied by Pierce. Cytosolic proteins (50 μg/lane) and molecular weight markers (Bio-Rad) were separated by SDS-polyacrylamide gel electrophoresis (running buffer: 25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.4) using 15% polyacrylamide gels (1 mm × 8.8 cm × 8.1 cm) (Sigma Chemical). After separation, protein was transferred to nitrocellulose membranes. For 1 h at 25 V (transfer buffer: 12 mM Tris base, 96 mM glycine, 20% methanol). Each membrane was blocked for 1 h at room temperature in 1% BSA in PBS-T. After blocking, the membranes were incubated in the presence of SULT1B1 antiserum or preimmune serum (1:100 in PBS-T) for 1 h with gentle rocking. Each blot was then washed in PBS-T (three washes, 5 min/wash). The blots were incubated in the presence of secondary antibody (1:3,000, goat anti-rat: Gibco BRL, Grand Island, NY) for 30 min with gentle rocking. The blots were washed in PBS-T (15 min) and then in PBS (three washes of 15 min each). Detection of antibody interaction was through enhanced chemiluminescence detection (ECL; Amersham, Arlington Heights, IL). Blots were exposed to X-ray film (X-OMAT AR; Kodak) for 30 s. The film was developed and examined by densitometric analysis using a personal densitometer (Molecular Dynamics) and quantified with Image Quant software.

**Statistical Analysis**

Data (four rats per group for the ontogeny studies, 6 rats per group for the DEX and PCN studies, and 4 rats per group for the studies with IX rats) were analyzed by one-way ANOVA followed by Duncan’s post hoc test. The accepted level of statistical significance was set at P < .05.

**Results**

The developmental expression of SULT1B1 mRNA was assessed in male and female rats (Fig. 1). Expression of SULT1B1 mRNA is low in male rats until 15 days of age. However, between 15 and 30 days of age, there is a dramatic increase in SULT1B1 expression (~6 fold), which is maximal by 30 to 45 days in male rats. Expression of SULT1B1 mRNA gradually decreased in male rats until 90 days of age.

The developmental expression of SULT1B1 was low in
females less than 30 days of age but increased about 2-fold from 30 to 45 days of age. Expression of SULT1B1 mRNA in female rats was steady from 45 to 90 days of age.

The influence of pituitary hormones on SULT1B1 mRNA expression was examined (Fig. 2). HX resulted in a slight decrease in SULT1B1 mRNA expression in both sexes, but the decrease was not statistically significant. Replacement of GH by either male or female pattern did not affect SULT1B1 expression from that observed in HX rats.

The influence of sex steroid hormones on SULT1B1 expression in HX rats was assessed. Replacement of EB, PR, or TP did not result in significant changes in expression from control or HX male and female rats (Fig. 3).

The influence of the steroidal compounds DEX and PCN was examined in pituitary-intact male and female rats (Fig. 4). Treatment of male rats with a single dose of 50 mg/kg DEX resulted in an approximate 4-fold increase in SULT1B1 mRNA expression. Similarly, PCN (75 mg/kg) induced SULT1B1 expression in male rats about 3-fold over control. The steroidal compounds were also administered to female rats. DEX was ineffective at inducing SULT1B1 mRNA in female rats. PCN treatment, however, did result in a 2-fold enhancement of SULT1B1 mRNA expression over control rats.

Peptide-specific antibodies were developed to enable detection of SULT1B1 protein. SULT1B1 immunoreactive protein was detected in both male and female rats (Fig. 5). Comparison of the expression of SULT1B1 protein between control and DEX- and PCN-treated rats revealed that DEX induced SULT1B1 protein in male rats but not female rats. PCN, however, did not induce of SULT1B1 protein in either male or female rats.

**Discussion**

In this study, we assessed the ontogenic expression and hormonal regulation of SULT1B1 mRNA and protein expression. Studies of SULT gene regulation have revealed that the expression of these enzymes is sex dependent in rats, with the phenol SULTs being predominant in male rats and hydroxysteroid SULTs being predominant in female rats. In addition, it has also been demonstrated that pituitary hormones (e.g., GH) exert control over SULT gene expression and that sex-specific secretion of GH (pulsatile in males and...
continuous in females) is important for the expression of most SULT isoforms (Liu and Klaassen, 1996a,b).

The ontogenic expression of SULT1B1 is unique among SULT isoforms. In contrast to other SULT isoforms, SULT1B1 is expressed at similar levels in adult male and female rats, despite differences in the developmental expression pattern. Other SULT1 family members (SULT1A1, SULT1C1, and SULT1E2) are expressed predominantly in adult male rats with virtually no expression of SULT1C1 or SULT1E2 in adult female rats (Liu and Klaassen, 1996a,b).

Although the expression of SULT1B1 is similar in adult male and female rats, the developmental expression profile of SULT1B1 mRNA is different between male and female rats; male rats exhibit higher levels of expression between 30 and 60 days of age than female rats but return to female levels at 60 days of age.
The lack of a sex difference in SULT1B1 expression suggests that this isoform has a parallel role in both sexes of rats. SULT1B1 has enzymatic activity toward thyroid hormone substrates T₃ and T₄ (Sakakibara et al., 1995; Fujita et al., 1997). Another SULT1 family isoform, SULT1C1, also has activity toward thyroid hormones. The findings reported here and by Fujita et al. (1997) imply that SULT1B1 may be more important than SULT1C1 for thyroid hormone homeostasis because SULT1C1 is almost exclusively expressed in male rats and because thyroid hormone does not exhibit sex-specific regulation.

The hormonal regulation of SULT1B1 mRNA expression is also distinct from other SULT isoforms. Phenol SULT and hydroxysteroid SULT gene expression are under the control of numerous hormones, including GH, certain sex steroids, and thyroid hormones. Runge-Morris and Wilusz (1984) demonstrated that hydroxysteroid SULT40/41 is negatively regulated by 3-methylcholanthrene. Thus, regulation of SULT expression is complex. SULT1B1 gene regulation is distinct from the other rat SULTs in that it is not dramatically altered by HX. Replacement of GH, which restores sex-specific SULT expression of certain isoforms (Liu and Klaassen, 1996a,b) does not affect SULT1B1 mRNA expression in HX rats. In addition, sex steroids do not appear to be major regulatory hormones of SULT expression and do not alter SULT1B1 gene expression.

SULT gene expression can be altered by pharmacologic chemicals. The synthetic glucocorticoid DEX has been shown to alter SULT mRNA expression and enzyme activity (Liu and Klaassen, 1996c). In addition, the antiglucocorticoid compound PCN induces SULT mRNA expression and enzyme activity (Liu and Klaassen, 1996c). Both DEX and PCN are known to induce expression of the cytochrome P-450 3A subfamily (Elshourbagy et al., 1981; Heuman et al., 1982; Scheutz et al., 1984). Dexamethasone induced SULT1A1 mRNA in both male and female rats and had isoform-specific effects on expression of hydroxysteroid SULTs in male and female rats. SULT1B1 mRNA, like SULT1A1, was markedly inducible (5-fold) by DEX in male rats, and the marked induction of SULT1B1 mRNA was reflected by a subsequent significant induction of SULT1B1 protein. However, in contrast to the other phenol SULTs, SULT1B1 mRNA was also inducible by PCN in male rats. SULT1B1 mRNA was not inducible by DEX in female rats, but PCN did elicit a small (2-fold) but significant increase in SULT1B1 mRNA. The smaller increase in SULT1B1 mRNA caused by PCN was not mimicked at the protein level.

In conclusion, SULT1B1 is unique among the phenol SULT family of enzymes (SULT1 family). SULT1B1 is present at similar levels in both adult male and female rats and does not exhibit sex-specific expression like other SULT isoforms. SULT1B1 is not regulated by hormones that are controlled at the level of the pituitary. Despite the differences in physiological hormone control of SULT1B1, this isoform is inducible by the synthetic glucocorticoid DEX (male rats). SULT1B1 inducibility by synthetic glucocorticoids (e.g., DEX) implies that endogenous glucocorticoids may play a role in the control of SULT1B1 expression, and this possibility is currently under investigation.

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References

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