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Molecular Cloning, Expression, and Characterization of a Novel Mouse Liver SULT1B1 Sulfotransferase[1]

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A mouse liver homogenate was shown to contain enzymatic activities catalyzing the sulfation of 3,4-dihydroxyphenylalanine (Dopa) and tyrosine isomers with a pH optimum of 8.25. Western blot analysis revealed a 34 kDa protein exhibiting immunologic cross-reactivity to antiserum against rat liver SULT1B1 sulfotransferase. By employing the reverse transcriptase-polymerase chain reaction (RT-PCR) technique, a 910-base pair product encoding the putative mouse liver SULT1B1 sulfotransferase was obtained. Using this PCR product as a probe, a cDNA containing the entire open reading frame of the mouse liver SULT1B1 sulfotransferase was cloned from a mouse liver Lambda ZAP cDNA library. The nucleotide sequence indicated it is a new enzyme. The deduced amino acid sequence exhibited 87.6, 72.3, 55.9, 54.2, 52.8, 51.1, and 49.4% identity to the amino acid sequences of the rat liver SULT1B1 sulfotransferase, human thyroid hormone sulfotransferase, mouse phenol sulfotransferase, rat liver phenol sulfotransferase, rat liver hydroxyarylamine sulfotransferase, mouse estrogen sulfotransferase, and rat estrogen sulfotransferase. Upon transfection of COS-7 cells with an expression vector (pcDNA3) harboring the cDNA encoding this new enzyme, a 34 kDa protein exhibiting immunologic cross-reactivity to antiserum against the rat liver SULT1B1 sulfotransferase was expressed. The recombinant sulfotransferase exhibited enzymatic activities toward Dopa and tyrosine isomers, as well as dopamine and 3,3′,5-triiodo-L-thyronine. Northern blot analyses indicated the SULT1B1 sulfotransferase was predominantly expressed in liver, but not in the other ten mouse organs examined. Furthermore, the enzyme was found to be expressed in a developmental stage-dependent manner, being at a very low level in liver samples from 1-day-old mice and then gradually increasing to the maximum level in liver samples from 4-week-old mice.

Key words: Dopa, molecular cloning, mouse liver, sulfotransferase, tyrosine.

Sulfation represents an important mechanism *in vivo* for the biotransformation and/or excretion of xenobiotics and endogenous compounds such as thyroid and steroid hormones, catecholamines, and bile acids (*1-3*). As another example of the latter group of compounds, free tyrosine-*O*-sulfate (TyrS) was first shown to be excreted in human urine in 1954 (*4*). Following a 40-year controversy over its mechanism of generation, we recently demonstrated unequivocally the occurrence of the sulfation of L-*p*-tyrosine in several mammalian cell lines (*5, 6*), as well as rat liver (*7*). Using HepG2 human hepatoma cells as a model, we have further shown the enzymatic sulfation of Dopa and other tyrosine isomers (*8*). A novel enzyme, initially designated as Dopa/tyrosine sulfotransferase and now renamed SULT1B1 sulfotransferase based on the newly recommended sulfotransferase nomenclature, was subsequently purified from rat liver and demonstrated, through cDNA cloning/sequencing, to be distinct from the other rat aryl sulfotransferases previously identified (*7*). In view of its unique activities toward Dopa and tyrosine isomers, this novel Dopa/tyrosine-sulfating enzyme has been hypothesized to play a role in the homeostatic regulation of Dopa and tyrosine *in vivo, i.e.*, the enzyme may serve to convert excess Dopa and tyrosine to their more highly water-soluble sulfated forms, thereby facilitating their excretion from the body. Since Dopa and tyrosine are used as biosynthetic precursors of catecholamines (including dopa-

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Abbreviations: Dopa, 3,4-dihydroxyphenylalanine; TyrS, tyrosine-*O*-sulfate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ATP, adenosine 5′-triphosphate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; TLC, thin-layer chromatography.

mine, norepinephrine, and epinephrine), that function as neurotransmitters or hormones in mammals, the SULT1B1 sulfotransferase is presumably important for normal functioning of the nervous system and/or the endocrine system. While proposing the role of the SULT1B1 sulfotransferase in the homeostatic regulation of Dopa and tyrosine, however, it is important to point out that the enzyme may also have other functions. For example, both our previous study (*7*) and that performed by Yamazoe *et al.* (*9*) indicated that the SULT1B1 sulfotransferase can catalyze the sulfation of thyroid hormones and other phenolic compounds.

In contrast to the enzymatic properties of the rat liver SULT1B1 sulfotransferase (*7*), the Dopa/tyrosine-sulfating sulfotransferase activities detected in HepG2 cells, interestingly, were found to be manganese-dependent (*8*). When HepG2 cytosol was subjected to DEAE Bio-Gel and hydroxylapatite column chromatography, the Dopa/tyrosine-sulfating sulfotransferase activities co-eluted closely with the human monoamine (M)-form phenol sulfotransferase (PST) (*8*). Furthermore, the HepG2 Dopa/tyrosine-sulfating sulfotransferase activities exhibited properties similar to those of the M-form PST in terms of thermostability and sensitivity to 2,6-dichloro-4-nitrophenol (*8*). By employing the PCR technique, we recently cloned the M-form PST, transiently expressed it in COS-7 cells, and showed that the recombinant M-form PST exhibited manganese-dependent Dopa/tyrosine-sulfating sulfotransferase activities (*10*). This new finding raised the question of whether or not there are multiple aryl sulfotransferases capable of catalyzing the Dopa/tyrosine sulfation in mammalian cells. It should be pointed out that sequence analysis revealed the deduced amino acid sequence of the human M-form PST cDNA exhibited only 72.6/52.4% similarity/identity to that of the rat liver SULT1B1 sulfotransferase (*7*). It is possible that there is a distinct sulfotransferase, orthologous to the rat liver SULT1B1 sulfotransferase, which is capable of catalyzing the sulfation of Dopa and tyrosine in a manganese-independent manner in HepG2 cells and/or other human cells. While the search for a human orthologue of the rat liver SULT1B1 sulfotransferase is still underway, we have decided to identify and characterize the enzyme(s) that catalyze(s) the sulfation of Dopa and tyrosine isomers in mouse liver.

In this paper, we report the characterization of the Dopa/tyrosine-sulfating sulfotransferase activities in a mouse liver homogenate, and the molecular cloning, expression, and characterization of a novel mouse liver SULT1B1 sulfotransferase, as well as the tissue-specific and developmental stage-dependent expression of this new enzyme.

MATERIALS AND METHODS

Materials—L-Dopa, D-Dopa, L-*para* (*p*)-tyrosine, D-*p*-tyrosine, DL-meta (*m*)-tyrosine, DL-ortho (*o*)-tyrosine, ninhydrin, aprotinin, antipain, benzamidine, soybean trypsin inhibitor, phenylmethylsulfonyl fluoride (PMSF), adenosine 5′-triphosphate (ATP), adenosine 5′-monophosphate (5′-AMP), sodium dodecyl sulfate (SDS), diethyl pyrocarbonate (DEPC), 2-(*N*-morpholino)ethanesulfonic acid (Mes), 3-(*N*-morpholino)propanesulfonic acid (Mops), *N*-2-hydroxylpiperazine-*N*′-2-ethanesulfonic acid (Hepes), 3-[*N*-tris-(hydroxymethyl)methylamino]propanesulfonic acid (Taps), 3-[dimethyl(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid (Ampso), and 2-(*N*-cyclohexylamino)ethanesulfonic acid (Ches) were products of Sigma Chemical Company. L-*p*-TyrS, DL-*m*-TyrS, dopamine-*O*-sulfate (dopamineS), and a mixture of L-Dopa 3-*O*-sulfate and L-Dopa 4-*O*-sulfate (collectively abbreviated as DopaS) were synthesized according to the procedures developed by Jevons (*11*). The mouse liver Lambda ZAP cDNA library and XL1-Blue MRF′ *Escherichia coli* host strain were products of Stratagene. The I.M.A.G.E. cDNA clone, 721406 (GenBank Accession number, AA267283), was obtained from Genome Systems. The pcDNA3 mammalian expression vector was a product of Invitrogen. The SuperScript Preamplification System, Random Primers DNA Labeling System, and LipofectAMINE were from Life Technologies. The TRI Reagent was a product of Molecular Research Center. *Taq* polymerase was purchased from Perkin Elmer. The *Taq* dye primer cycle sequencing kits were from Applied Biosystems. All restriction endonucleases were from New England Biolabs. Various organs from a 2-month-old male C57BL6 mouse and livers from male C57BL6 mice of different ages were purchased from Harlan Bioproducts. Carrier-free sodium [^{35}S]sulfate (3,000 Ci/mM) and Ecolume were from ICN Biomedicals. [α-^{35}S]-Deoxyadenosine-5′-(α-thio)triphosphate (dATP) and [α-^{32}P]deoxycytidine-5′-triphosphate (dCTP) were products of DuPont. The hybond N+ nylon membrane filters and ECL Plus Western blotting detection system were from Amersham. The COS-7 SV40-transformed African green monkey kidney cells (ATCC CRL 1651) were from the American Type Culture Collection. Chromatogram cellulose thin-layer chromatography (TLC) plates were from Eastman Kodak Company. Rabbit antiserum against the rat liver SULT1B1 sulfotransferase was prepared as previously described (*7*). All other chemicals were of the highest grade commercially available.

Preparation of a Mouse Liver Homogenate—Mouse liver (1.5 g), rinsed thoroughly with ice-cold phosphate-buffered saline (PBS) and suspended in 3 ml of buffer A comprising 10 mM Tris-HCl (pH 7.4), 250 mM sucrose, 10 μg/ml aprotinin, and 1 mM PMSF, was homogenized using a Tekmar Tissumizer. The homogenized preparation was centrifuged at 10,000 × *g* for 10 min at 4°C, and the resultant supernatant was used in the enzymatic assays described below.

Enzymatic Assay—The Dopa/tyrosine-sulfating sulfotransferase activities were assayed using PAP[^{35}S] as the sulfate donor. The standard assay mixture, with a final volume of 30 μl, contained 50 mM Taps-NaOH (pH 8.25), 250 mM sucrose, 25 mM NaF, 1 mM 5′-AMP, 30 μg/ml aprotinin, 14 μM PAP[^{35}S] (15 Ci/mmol), and 1 mM substrate (Dopa, tyrosine, *etc.*). The reaction was started by the addition of the enzyme preparation, allowed to proceed for 60 min at 37°C, and terminated by heating at 100°C for 3 min. The precipitate formed was cleared by centrifugation. The clear supernatant was subjected to analysis of the [^{35}S]sulfated product as described below. In some reactions, 10 mM $MnCl_2$ was included in the reaction mixture to examine the effect of manganese ions on the Dopa/tyrosine-sulfating sulfotransferase activities.

Analysis of [^{35}S]Sulfated Compounds—For the analysis of Tyr[^{35}S], Dopa[^{35}S], dopamine[^{35}S], or other [^{35}S]-sulfated products, 3 μl of the clear reaction mixture was

mixed with 1 μg of a synthetic standard (TyrS, DopaS, or dopamineS), spotted onto a 10 × 10 cm cellulose TLC plate, and then analyzed according to the two-dimensional thin-layer separation procedure previously developed (*12*). Briefly, the plate was first subjected to high-voltage electrophoresis (1,000 V, 70 min) in 7.8% (v/v) acetic acid/2.5% (v/v) 88% formic acid, pH 1.9. After electrophoresis, the plate was air-dried and then subjected, in the second dimension, to ascending chromatography in *n*-butanol/88% formic acid/isopropanol/H_2O (3:1:1:1, by volume). Upon completion of the chromatography, the plate was sprayed with a ninhydrin solution (0.5% in acetone). The ninhydrin-stained spot of the sulfated product was scraped off, suspended in 0.5 ml H_2O, and then mixed with 4 ml of Ecolume, a scintillation cocktail. The radioactivity associated with Tyr[^{35}S], Dopa[^{35}S], dopamine[^{35}S], or another [^{35}S]sulfated product was counted.

Cloning of Mouse Liver SULT1B1 Sulfotransferase cDNAs—The RT-PCR technique was employed to prepare the DNA probe used for the cDNA library screening. Total RNA was isolated from a liver specimen from 2-month-old male C57BL6 mouse using the TRI Reagent according to the manufacturer's instructions. The first strand cDNA was synthesized using the SuperScript Preamplification system with the total mouse liver RNA as the template and oligo(dT) as the primer. With the first strand cDNA as the template, a PCR reaction, performed in a 50 μl reaction mixture containing the *Taq* polymerase, was carried out with 5′-CTACAAAAATGGGTACTGCAGAAGA-3′ as the sense primer and 5′-GTTTAGGCACTCTGAATATCTGT-GC-3′ as the antisense primer. The amplification conditions were 15 cycles of 45 s at 94°C, 1 min at 55 to 40°C (touch down; 1°C decrease/cycle), and 1 min at 72°C, plus 20 more cycles of 45 s at 94°C, 1 min at 40°C, and 1 min at 72°C. The reaction mixture was applied to a 2% agarose gel, separated by electrophoresis, and then visualized by ethidium bromide staining. A major 910-nucleotide PCR product was detected and excised from the gel, and the DNA was isolated by spin filtration. Upon verification of the sequence by cycle sequencing, the purified PCR product was subcloned into the *Eco*RV site of pBluescript SK(−) and then transformed into *E. coli* XL1-Blue MRF′. The recombinant plasmid was purified using the alkaline/SDS method, and the PCR product insert was cut out by digestion with *Eco*RI and *Hin*dIII. The insert DNA was purified by agarose gel electrophoresis followed by spin filtration. The purified insert DNA was labeled with [α-^{32}P]dCTP using random primers (*13*), and the labeled DNA was used as the probe for screening the cDNA encoding the SULT1B1 sulfotransferase in a mouse liver Lambda ZAP cDNA library. Approximately 2×10^6 plaques from the library were screened with the ^{32}P-labeled DNA probe by hybridization on Hybond N+ nylon membrane filters. Nylon membrane filter replicas of plaques/XL1-Blue MRF′ grown on 150 mm Petri dishes, upon prehybridization for 2 h at 65°C, were hybridized with the ^{32}P-labeled DNA probe overnight at 65°C. The hybridized membranes were washed once with 2 × SSC plus 0.1% SDS at room temperature and twice with 0.1 × SSC plus 0.1% SDS at 65°C, followed by autoradiography to reveal the positive cDNA clones.

DNA Sequence Determination and Analysis—The positive cDNA clones were subjected to double-stranded sequencing according to the cycle sequencing method using *Taq* dye primer cycle sequencing kits with the T3 or T7 primer. The nucleotide sequence, as well as the deduced amino acid sequence, of the cloned cDNA was analyzed using the E-mail servers at NCBI and EMBL for sequence homology to other known aryl sulfotransferases.

Transient Expression of the Mouse SULT1B1 Sulfotransferase in COS-7 Cells—COS-7 cells, normally maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, were used as the host cells for expression of the enzyme. Dishes (60 mm) of COS-7 cells were individually transfected with 2 μg of the pcDNA3 vector only or a pcDNA3 derivative harboring the mouse SULT1B1 sulfotransferase sequence using the Lipofect-AMINE-mediated procedure. Incubation was performed for 18 h at 37°C, according to the manufacturer's instructions. The transfected cells were incubated at 37°C in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. At the end of the 48-h incubation, the cells were rinsed twice with PBS, and then homogenized in the buffer A comprising 10 mM Tris-HCl (pH 7.4), 250 mM sucrose, 10 μg/ml aprotinin, and 1 mM PMSF. Aliquots of the homogenates prepared were assayed for Dopa/tyrosine-sulfating sulfotransferase activities.

Northern Blot Analysis—Frozen mouse organ samples or liver samples from mice of different ages (~100 mg per sample) were directly placed in 1 ml aliquots of the TRI Reagent and then homogenized immediately using a Tekmar Tissumizer pre-soaked overnight in DEPC-treated water. Total RNAs were prepared according to the manufacturer's instructions. Ten microliter aliquots of RNA (1 mg/ml) samples solubilized in a sample buffer (53% formamide, 5.8% formaldehyde, 0.025 M Mops, 6.3% glycerol, and 0.5% bromophenol blue), preincubated at 65°C for 15 min, were electrophoresed on a formaldehyde (2.2 M) agarose gel (1.2%). After electrophoresis, the RNAs were capillary-transferred to a Hybond N+ nylon membrane for 2 h with 50 mM NaOH as the transfer solution. Afterwards, the membrane was air-dried, prehybridized at 65°C for 1 h in a prehybridization solution (5 × Denhardt, 0.5% SDS, 6 × SSC, and 100 μg/ml salmon sperm DNA), and then hybridized with the mouse liver SULT1B1 sulfotransferase cDNA, labeled with [α-^{32}P]dCTP by random priming (*13*). After overnight hybridization at 65°C, the membrane was washed once in 2 × SSC containing 0.1% SDS for 1 h at room temperature and then twice in 0.1 × SSC containing 0.1% SDS for 45 min at 65°C, followed by autoradiography at −80°C with an intensifying screen.

Western Blot Analysis—The samples to be analyzed were first subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 12% polyacrylamide gel according to the method of Laemmli (*14*). After electrophoresis, proteins separated in the polyacrylamide gel were electrotransferred onto an Immobilon-P membrane at a constant of 200 mA for 4 h in a buffer solution comprising 25 mM Trizma base and 192 mM glycine (*15*). The blotted membrane was blocked with 5% non-fat dried milk in PBS for 1 h and then probed with 20 μl of anti-rat liver SULT1B1 sulfotransferase antiserum. After 1-h incubation, the membrane was washed with PBS, treated with horseradish peroxidase conjugated secondary antibodies in PBS containing 5% non-fat dried milk, and then processed using the ECL Plus non-radioactive detection system according to the manufacturer's instructions. Autoradiography was

performed on the processed membrane.

Miscellaneous Methods—PAP[^{35}S] (15 Ci/mmol) was synthesized from ATP and [^{35}S]sulfate using the sulfate-activating enzymes, ATP sulfurylase and adenosine 5′-phosphosulfate kinase, from *Bacillus stearothermophilus*, as described previously (*16*). Protein determination was performed according to the method of Bradford (*17*), with bovine serum albumin as the standard.

RESULTS

Preliminary Characterization of the Mouse Liver SULT1B1 Sulfotransferase—The Dopa/tyrosine-sulfating sulfotransferase activities present in a mouse liver homogenate were characterized as described below.

pH Optimum and Effects of Manganese Ions—For determining the pH optimum of the Dopa/tyrosine sulfotrtansferase activities, D-Dopa was used as the substrate in order to avoid decarboxylation by the aromatic L-amino acid decarboxylase present in the mouse liver homogenate. As shown in Fig. 1, the pH optimum of the mouse liver SULT1B1 sulfotransferase was determined to be around pH 8.25. In contrast to our previous findings on the stimulation of the Dopa/tyrosine-sulfating sulfotransferase activities in a HepG2 human hepatoma cell homogenate (*5, 9*), the addition of 10 mM $MnCl_2$ resulted in a dramatic decrease in the Dopa/tyrosine-sulfating sulfotransferase activities with either Dopa or tyrosine isomers as substrates (data not shown).

Dopa/Tyrosine-Sulfating Activities toward Dopa and Tyrosine Isomers—Different Dopa and tyrosine isomers were examined by means of the standard assay described under "MATERIALS AND METHODS." As shown in Table I, both L-Dopa and D-Dopa gave much higher specific activities than those obtained using L-*p*-tyrosine or D-*p*-tyrosine. The specific activity obtained with DL-*m*-tyrosine as the substrate was 55.2 and 17.2 times those obtained with L-*p*-tyrosine and D-*p*-tyrosine as the substrate, respectively. No activities could be detected with DL-*o*-tyrosine as the substrate.

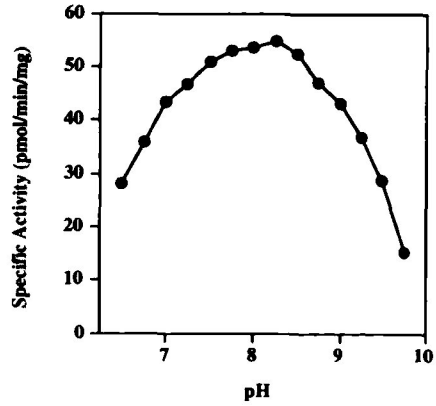

Fig. 1. **pH-dependence of the mouse liver Dopa/tyrosine-sulfating sulfotransferase activities.** The Dopa/tyrosine sulfotransferase assays were carried out under the standard assay conditions with D-Dopa as the substrate as described under "MATERIALS AND METHODS" except for the different buffers used. The buffers used were 50 mM Mes at pH 6.5; Mops at pH 6.75, 7.0, or 7.25; Hepes at pH 7.5, 7.75, or 8.0; Taps at pH 8.25, or 8.5; Ampso at pH 8.75, 9.0, or 9.5; and Ches at 9.75. Data represent calculated mean values for three experiments.

Immunologic Cross-Reactivity toward Antiserum against the Rat Liver SULT1B1 Sulfotransferase—Western blot analysis was performed to examine the immunologic cross-reactivity of the mouse SULT1B1 sulfotransferase toward antiserum against the rat liver SULT1B1 sulfotransferase. Since this antiserum had previously been shown to be capable of recognizing both the native and SDS-denatured forms of the enzyme, we decided, in an attempt to minimize non-specific immunologic cross-reactivity, to immunoprecipitate the mouse SULT1B1 sulfotransferase from the mouse liver homogenate prior to Western blot analysis. As shown in Fig. 2, for the mouse liver homogenate sample immunoprecipitated using the antiserum against the rat liver SULT1B1 sulfotransferase, a 34 kDa protein exhibiting immunologic cross-reactivity toward the antiserum was detected (lane 2). Probably due to the heterologous antiserum used, several minor contaminating protein species, in particular a 32 kDa protein, were also detected. The 34 kDa protein, nevertheless, exhibited the strongest band intensity and migrated to the same electrophoretic position as the control rat liver SULT1B1 sulfotransferase. This finding stimulated our interest in PCR-cloning of the putative mouse SULT1B1 sulfotransferase sequence using 5′- and 3′-primers based on the cDNA sequence of the rat enzyme.

Molecular Cloning of the Mouse Liver SULT1B1 Sul-

TABLE I. **Specific activities of the mouse liver SULT1B1 sulfotransferase with different Dopa and tyrosine isomers as substrates.**

Substrate	Specific activity (pmol/min/mg)[a]	% of L-Dopa[b]
L-Dopa	28.76±0.45	100
D-Dopa	54.85±1.75	190.7
L-*p*-Tyrosine	0.19±0.03	0.7
D-*p*-Tyrosine	0.61±0.17	2.1
DL-*m*-Tyrosine	10.49±0.26	36.5

[a]Specific activity refers to activity per mg protein of the mouse liver homogenate. Data shown represent means±SD for three experiments. [b]Relative activity compared to that of L-Dopa as 100%.

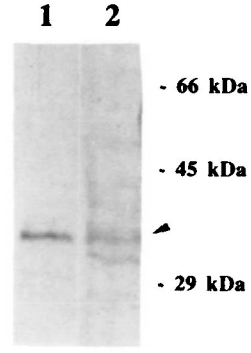

Fig. 2. **Western blot analysis of the mouse liver protein(s) exhibiting immunologic cross-reactivity toward antibodies against the rat liver SULT1B1 sulfotransferase.** The figure shows the autoradiograph of the Immobilon-P membrane used in the Western blot analysis. The samples analyzed were: rat liver cytosol (lane 1) and mouse liver cytosol immunoprecipitated with anti-rat liver SULT1B1 sulfotransferase antiserum (lane 2).

fotransferase—To amplify the putative mouse liver SULT1B1 sulfotransferase sequence, a RT-PCR reaction involving the first-strand cDNA prepared from the total mouse liver RNA as the template in conjunction with two primers (5′-CTACAAAAATGGGTACTGCAGAAGA-3′ and 5′-GTTTAGGCACTCTGAATATCTGTGC-3′) corresponding to residues −8 through +17 and residues +877 through +902 of the nucleotide sequence of the rat liver SULT1B1 sulfotransferase cDNA (*7*) was first carried out. A specific 910-base pair PCR product was obtained. Nucleotide sequencing of this PCR product revealed it exhibited greater than 87% sequence homology to the rat liver SULT1B1 sulfotransferase (data not shown). Using this PCR product as a probe for screening, eighteen positive cDNA clones were subsequently obtained from the mouse liver Lambda ZAP cDNA library. pBluescript phagemids carrying these positive cDNA clones were prepared by *in vivo* excision. One of them, demonstrated to encompass the entire open reading frame by PCR screening using the two primers described above, was subjected to complete nucleotide sequencing. The nucleotide sequence of this mouse liver SULT1B1 sulfotransferase cDNA, which corresponds to nucleotide residues −48 through +911 of the nucleotide sequence shown in Fig. 3, contains only 48 residues in its 5′-untranslated region and a short 11-nucleotide 3′-untranslated sequence. Examining the sequences stored in the expressed sequence tag (EST) database (*18*), we have identified a mouse sulfotransferase cDNA clone (GenBank Accession number, AA267283) which contains a partial 5′-nucleotide sequence identical to that of the SULT1B1 sulfotransferase cDNA we isolated from the mouse liver cDNA library. Since this EST database clone contains longer 5′- and 3′-untranslated sequences than the one we isolated, we decided to sequence it in order to obtain more sequence data on these two regions. Figure 3 shows the nucleotide and deduced amino acid sequences of this cDNA, which contains sequences identical to those determined for the mouse liver SULT1B1 sulfotransferase cDNA we cloned. The open reading frame encompasses 897 nucleotides and encodes a 299 amino acid polypeptide. The predicted molecular weight, 34,901, is in good agreement with the results (M_r = 34,000) of the Western blot analysis experiment described earlier. It should be pointed out that, although there is an additional methionine residue located 14 amino acid residues downstream from the N-terminus shown in Fig. 3, the translation initiation methionine

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GGCACGAGGGTGTCCATCTCAGGTCACCACCAACTCTGGCACGAGGGTGTCCATCTCAGG  -63

TCACCACCAACTCTGAAGGCTCTTCACAGCAGCATTCGTTCTGATTGAAGCCTGCTGCAA   -3

AAATGAGTGCCTCAGAAGACGTTTGGAGAAAAGATCTGAAGATGATCCATGGCTACCCCA   58
  M  S  A  S  E  D  V  W  R  K  D  L  K  M  I  H  G  Y  P  M

TGATCTATGCTTTTGCACTCAATTGGGAAAGGATTGAAGAGTTCCAGAGCACACCAGGTG  118
  I  Y  A  F  A  L  N  W  E  R  I  E  E  F  Q  S  T  P  G  D

ACATTGTAATAACCACTTACCCTAAATCAGGTACTACTTGGCTTAGTGAGATTGTAGACA  178
  I  V  I  T  T  Y  P  K  S  G  T  T  W  L  S  E  I  V  D  M

TGGTTCTAAATGATGGAAATGTTGAAAAATGTAAGAGAGATGTTATCACCTCCAAAGTTC  238
  V  L  N  D  G  N  V  E  K  C  K  R  D  V  I  T  S  K  V  P

CAATGTTGGAACTGAGTGTTCCTGGAATAAGAATATCAGGTGTTGAACTCTTGAAGAAAA  298
  M  L  E  L  S  V  P  G  I  R  I  S  G  V  E  L  L  K  K  T

CTCCATCACCTCGGATAATAAAGACACATCTTCCAATCGATCTACTCCCAAAATCCTTCT  358
  P  S  P  R  I  I  K  T  H  L  P  I  D  L  L  P  K  S  F  W

GGGAGAACAAGTGCAAGATGATTTACCTTGCTCGAAATGGCAAGGATGTTGCTGTCTCCT  418
  E  N  K  C  K  M  I  Y  L  A  R  N  G  K  D  V  A  V  S  Y

ATTATCATTTTGATCTGATGAATAGTATTAATCCTCTTCCTGGCACCTGGGAAGAATATC  478
  Y  H  F  D  L  M  N  S  I  N  P  L  P  G  T  W  E  E  Y  L

TGGAGAAATTCCTAGCTGGAAATGTGGCCTATGGTTCATGGTTTGATCATGTTAAGAGTT  538
  E  K  F  L  A  G  N  V  A  Y  G  S  W  F  D  H  V  K  S  W

GGTGGGAAAAGAGGGAAGAGCATCCTTTACTTTACTTATACTATGAAGAATTGAAACAGA  598
  W  E  K  R  E  E  H  P  L  L  Y  L  Y  Y  E  E  L  K  Q  N

ACCCAAAGAAAGAAATCAAGAAGATAGCCAGCTTTCTAGACAAGACCTTGGATGAAGAGG  658
  P  K  K  E  I  K  K  I  A  S  F  L  D  K  T  L  D  E  E  A

CCTTGGACAGGATCGTCCATCACACCTCCTTTGAAATGATGAAGGAAAACCCCCTGGTCA  718
  L  D  R  I  V  H  H  T  S  F  E  M  M  K  E  N  P  L  V  N

ATTACACCCATCTGCCCACAGCAATGATGGACCACAGCAAGTCCCCTTTCATGAGAAAAG  778
  Y  T  H  L  P  T  A  M  M  D  H  S  K  S  P  F  M  R  K  G

GTATTGTTGGGGACTGGAAAAATTACTTCACAATGACCCAAACTGAGCAATTTGATGCTG  838
  I  V  G  D  W  K  N  Y  F  T  M  T  Q  T  E  Q  F  D  A  V

TCTATAAGAAGAAGATGTCTGGAACAACACTTGAGTTCTGCACAGACATTCAGAGTGCCT  898
  Y  K  K  K  M  S  G  T  T  L  E  F  C  T  D  I  Q  S  A

AATCTACAACTTGAATATATGGTTTCTTAAAATAGTAACCTGGAAGAGAAATCAAATAGA  958

TTCATGAAGGAAAAATAAATGTGCTTTAAAAATGCTAATTGAAAACATACTACACATTCC 1018

CCAGCAGGTAATCTTCCAAATGATCTAGAGCCAAGGACTTTTGTTACCTTAGTTTTCAAA 1078

GGATATGTCTTCAGATTTCTAGATTCTTACTGAGTTGAATAAATACA               1125
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Fig. 3. **Nucleotide and deduced amino acid sequences of the mouse liver SULT1B1 cDNA.** Nucleotides are numbered in the 5′ to 3′ direction with the adenosine of the translation initiation codon designated as +1. The polyadenylation signals are underlined.

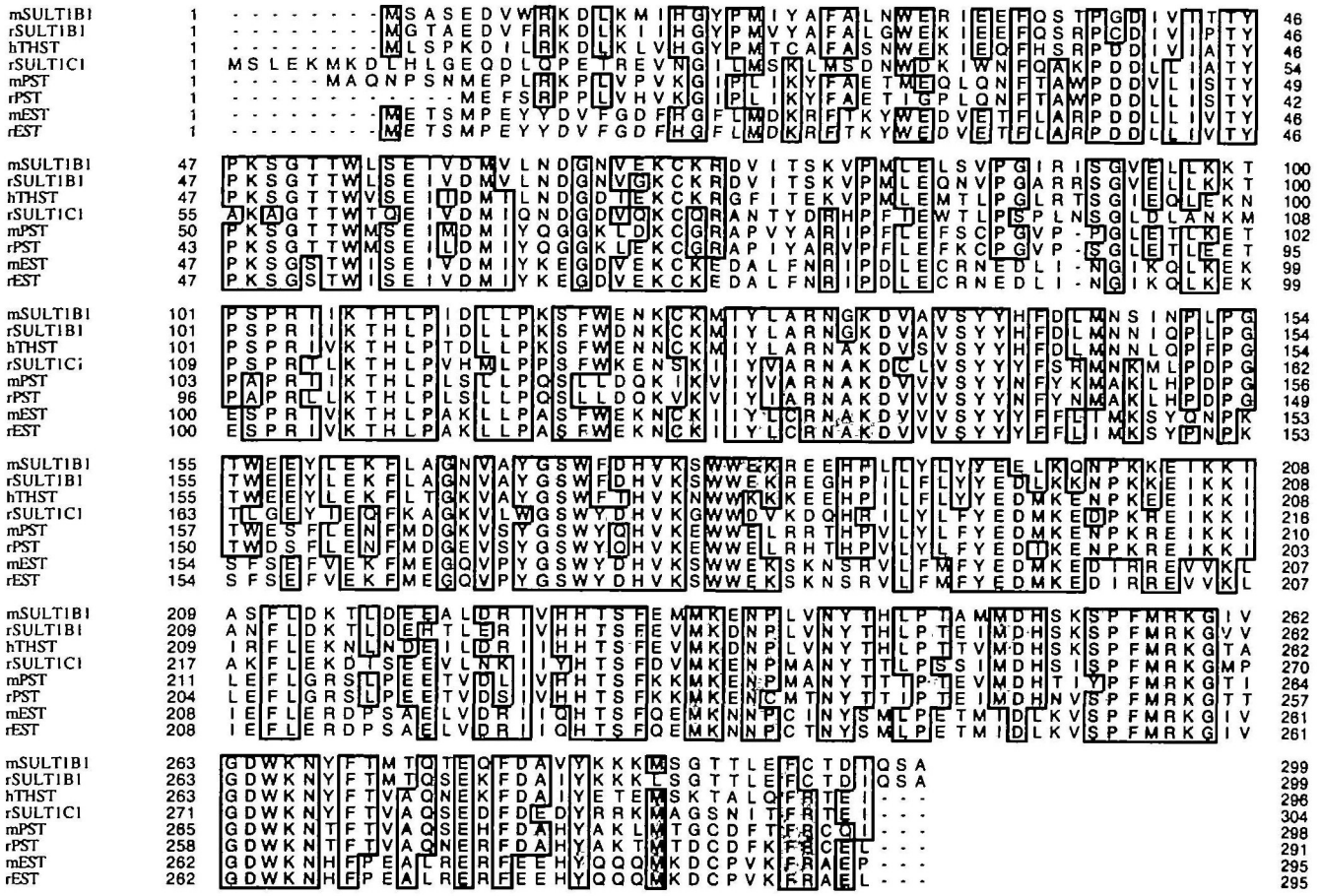

Fig. 4. **Amino acid sequence comparison of the mouse liver SULT1B1 sulfotransferase with rat liver SULT1B1 sulfotransferase (rSULT1B1), human thyroid hormone sulfotransferase (hTHST), rat liver hydroxyarylamine sulfotransferase (rSULT1C1), mouse liver phenol sulfotransferase (mPST), rat liver phenol sulfotransferase (rPST), mouse estrogen sulfotransferase (mEST), and rat estrogen sulfotransferase (rEST).** The sequences are aligned with the N-terminal methionine residue of the mouse liver SULT1B1 sulfotransferase designated as position 1. Residues conserved among at least five of the eight sulfotransferases are boxed in shadowed areas.

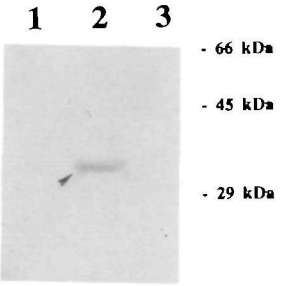

Fig. 5. **Expression of the recombinant mouse liver SULT1B1 sulfotransferase in COS-7 cells.** The figure shows the autoradiograph of the Immobilon-P membrane used in the Western blot analysis for the presence of the recombinant mouse liver SULT1B1 sulfotransferase. The samples analyzed were: untransfected COS-7 cell homogenate (lane 1), homogenate of COS-7 cells transfected with pcDNA3 harboring the cDNA encoding the mouse liver SULT1B1 sulfotransferase (lane 2), and homogenate of COS-7 cells transfected with pcDNA3 vector only (lane 3).

TABLE II. **Expression of the recombinant mouse liver SULT1B1 sulfotransferase in COS-7 cells.**

Substrate	Specific activity (fmol/min/mg protein)[a]	
	Mouse SULT1B1 ST[b]	Control[c]
L-Dopa	1,749±171	95.8±11.0
D-Dopa	2,274±38	196.6±1.7
DL-*m*-Tyrosine	896±19	16.1±0.3
Dopamine	8,531±393	778.7±33.0
3,3′,5-Triiodo-L-thyronine	6,054±576	167.5±11.3

[a]Specific activities shown represent means±SD for three experiments. [b]COS-7 cells transfected with pcDNA3 harboring the mouse SULT1B1 ST cDNA. [c]COS-7 cells transfected with pcDNA3 only.

residue was designated based on (i) the predicted molecular weight that matches the Western blot analysis result, and (ii) the sequence alignment on comparison of the rat SULT1B1 sulfotransferase and other known aryl sulfotransferases (see below). The termination codon, located at nucleotide residues 898-900, was followed by a 228-nucleotide 3′-untranslated sequence. Two polyadenylation signals (AATAAA) (*19*) were noted in the 3′-untranslated sequence. As shown in Fig. 4, the deduced amino acid sequence of the mouse liver SULT1B1 sulfotransferase cDNA exhibits 87.6, 72.3, 55.9, 54.2, 52.8, 51.1, and 49.4% identity to the amino acid sequences of the rat liver SULT1B1 sulfotransferase (*7*), human thyroid hormone

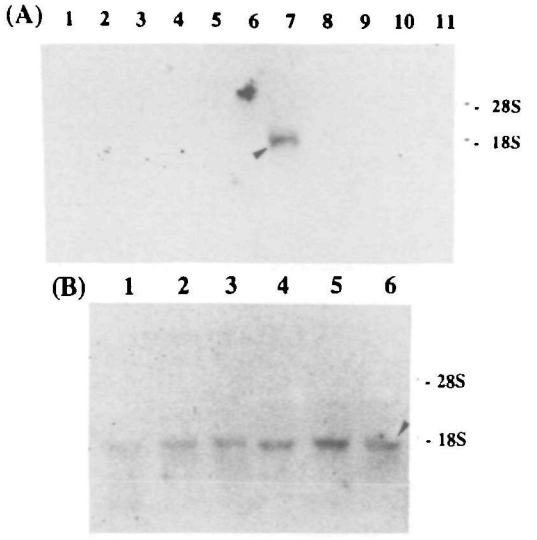

Fig. 6. **Northern blot analyses for the tissue-specific and developmental stage-dependent expression of the SULT1B1 sulfotransferase in mouse.** Part (A) shows the results of Northern blot analysis for the presence of the SULT1B1 sulfotransferase mRNA in different mouse organs. The total RNA samples (20 μg each) analyzed were prepared from: adrenal gland (lane 1), brain (lane 2), epididymis (lane 3), heart (lane 4), intestine (lane 5), kidney (lane 6), liver (lane 7), lung (lane 8), pancreas (lane 9), spleen (lane 10), and thyroid (lane 11). The figure shows an autoradiograph of the blot. The arrowhead indicates the position of the SULT1B1 sulfotransferase mRNA band. Part (B) shows the results of Northern blot analysis for the expression of the SULT1B1 sulfotransferase mRNA in liver samples prepared from rats of different ages. The RNA (20 μg) samples analyzed were prepared from 1-day-old (lane 1), 3-day-old (lane 2), 1-week-old (lane 3), 2-week-old (lane 4), 4-week-old (lane 5), and 8-week-old (lane 6) male rats. The arrowhead indicates the position of the SULT1B1 sulfotransferase mRNA band.

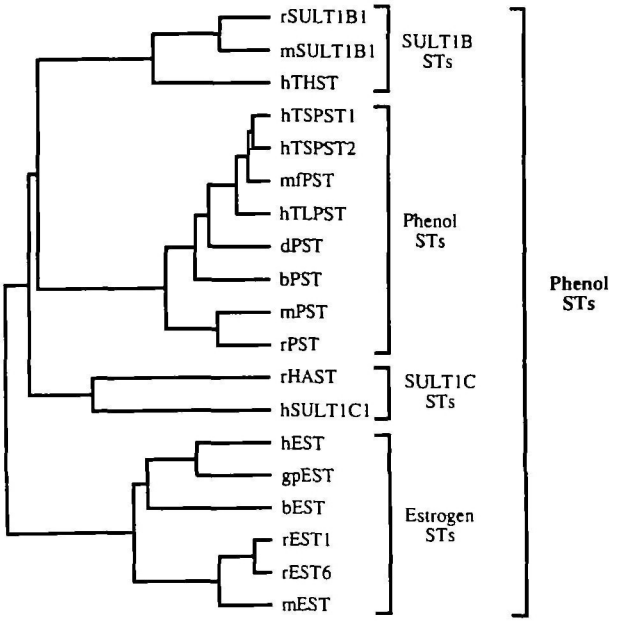

Fig. 7. **Deduced phylogeny of the cytosolic phenol sulfotransferase gene family with the unweighted pair-group method of analysis (UPGMA).** The enzymes listed include rat liver SULT1B1 sulfotransferase (rSULT1B1) (*7*), mouse liver SULT1B1 sulfotransferase (mSULT1B1), human thyroid hormone sulfotransferase (hTHST) (*20*), human thermostable phenol sulfotransferase-1 (hTSPST1) (*28*), human thermostable phenol sulfotransferase-2 (hTSPST2) (*36*), *Macaca fascicularis* phenol sulfotransferase (mfPST) (*37*), human thermolabile phenol sulfotransferase (hTLPST) (*29*), dog phenol sulfotransferase (dPST) (*38*), bovine phenol sulfotransferase (PST) (*39*), mouse phenol sulfotransferase (mPST) (*21*), rat phenol sulfotransferase (rPST) (*22*), rat hydroxyarylamine sulfotransferase (rHAST) (*23*), human SULT1C1 sulfotransferase (hSULT1C1) (*32*), human estrogen sulfotransferase (hEST) (*40*), guinea pig estrogen sulfotransferase (gpEST) (*41*), bovine estrogen sulfotransferase (bEST) (*42*), rat estrogen sulfotransferase-1 (rEST1) (*25*), rat estrogen sulfotransferase-6 (rEST6) (*43*), and mouse estrogen sulfotransferase (mEST) (*24*).

sulfotransferase (*20*), mouse liver phenol sulfotransferase (*21*), rat liver phenol sulfotransferase (*22*), rat liver hydroxyarylamine sulfotransferase (*23*), mouse estrogen sulfotransferase (*24*), and rat estrogen sulfotransferase (*25*) (based on analysis using the FASTA Sequence Similarity Search). It was noted that the mouse SULT1B1 sulfotransferase, similar to other sulfotransferase enzymes, contains the so-called "signature sequences" (YPKSGTxW and RKGxxGDWKNxFT) (*26*), which have been proposed to be involved in the binding of PAPS, a co-substrate for the sulfation reaction (*3*).

Expression and Characterization of the Recombinant Mouse Liver SULT1B1 Sulfotransferase—The recombinant protein was expressed in COS-7 cells, and then subjected to functional characterization and examination of its immunoreactivity toward antiserum against the rat liver SULT1B1 sulfotransferase. As shown in Fig. 5, a 34 kDa protein cross-reactive toward the antiserum against the rat SULT1B1 sulfotransferase was expressed specifically, when the COS-7 cells were transfected with an expression vector (pcDNA3) harboring the cDNA encoding the mouse liver SULT1B1 sulfotransferase. When the cell homogenates were assayed for Dopa/tyrosine-sulfating sulfotransferase activities, it was found that the sample prepared from the cells transfected with the expression vector harboring the cDNA exhibited highly elevated Dopa/tyrosine-sulfating sulfotransferase activities (Table II). The recombinant mouse SULT1B1 sulfotransferase was also found to be capable of catalyzing the sulfation of dopamine and 3,3′,5-triiodo-L-thyronine.

Tissue Specificity of the Expression of the Mouse SULT1B1 Sulfotransferase—The expression of the SULT1B1 sulfotransferase in different mouse organs was examined using the Northern blotting technique. As shown in Part (A) of Fig. 6, among the eleven mouse organs examined, the mRNA encoding the enzyme was found to be present predominantly in the liver, being nearly undetectable in the other ten mouse organs. Northern blot analysis was performed to examine the expression of the enzyme in the livers male mice of different ages. As shown in Part (B) of Fig. 6, using the mouse liver SULT1B1 sulfotransferase cDNA as the probe, the mRNA encoding the enzyme was found to be present at a very low level in liver samples from 1-day-old mice, and then gradually increased to the maximum level in male mice of older than 4 weeks.

DISCUSSION

Cytosolic sulfotransferases in mammalian animals are traditionally regarded as detoxifying enzymes that facili-

tate the removal of drugs and xenobiotic compounds through sulfate conjugation (*1*). Increasingly, however, these enzymes are being shown to be involved in the sulfation (or more precisely, sulfonation) of endogenous compounds, such as thyroid and steroid hormones, catecholamines, and bile acids, to fulfill fundamental biochemical/physiological needs (*27*). In human tissues, five cytosolic sulfotransferases, *i.e.* the "M-form" PST and two variant "P form" PSTs (*28, 29*), the dehydroepiandrosterone sulfotransferase (DHEAST) (*30*), and the estrogen sulfotransferase (EST) (*31*), have been identified. (Recently, two human sulfotransferase sequences (*32, 33*) exhibiting homology to the rat liver hydroxyarylamine sulfotransferase (see below) and two hydroxysteroid sulfotransferase (HSST) sequences (*34, 35*) were reported.) In rat tissues, two more sulfotransferases, the hydroxyarylamine sulfotransferase (HAST) (*23*) and the SULT1B1 sulfotransferase (*7, 9*), have been detected. Based on the results of sequence analysis, these different cytosolic sulfotransferases are classified into two major gene families (*9, 26*), the phenol sulfotransferase family and the hydroxysteroid sulfotransferase family. Currently, the phenol sulfotransferase family consists of four subfamilies, PST (ST1A), EST (ST1E), HAST (ST1C), and SULT1B ST (ST1B) (*26*; *cf.* Fig. 7). Since none of the reported mouse liver sulfotransferases has been classified into the latter two subfamilies, it is possible that the mouse liver may contain novel sulfotransferases that belong to the ST1B and/or ST1C subfamilies. We were therefore interested in searching for new mouse liver sulfotransferases.

Using a mouse liver homogenate, we first detected sulfotransferase activities catalyzing the sulfation of Dopa and tyrosine isomers. Western blot analysis was subsequently performed to reveal the presence of a 34 kDa protein exhibiting immunologic cross-reactivity toward antiserum against the 34 kDa rat liver SULT1B1 sulfotransferase. These results implied the presence of a similar SULT1B1 sulfotransferase in the mouse liver. Instead of performing the tedious purification procedure to prepare a purified enzyme for further characterization, our previous work for elucidation of the nucleotide sequence of the rat liver SULT1B1 sulfotransferase (*7*) provided an opportunity for direct cloning of the orthologous mouse liver enzyme. Since the 5′- and 3′-regions of the open reading frame of the rat liver SULT1B1 sulfotransferase cDNA appear to be rather unique in comparison with those of the other mammalian aryl sulfotransferases previously reported (*7*), we decided to first PCR-amplify the mouse SULT1B1 sulfotransferase sequence using oligonucleotide primers based on these regions. Indeed, RT-PCR using these two primers with the total mouse liver RNA as the template yielded a specific 910-base pair PCR product. Using this PCR product as a probe for screening, a cDNA containing the complete open reading frame was obtained from a mouse liver Lambda ZAP cDNA library. Analysis of the nucleotide sequence of this cDNA strongly indicated it is as a mouse orthologue of the rat liver (ST1B-type) SULT1B1 sulfotransferase. Its authenticity was further confirmed by the expression of a functionally active recombinant enzyme that cross-reacted with the antiserum against the rat liver SULT1B1 sulfotransferase. It should be pointed out that the highly conserved amino acid sequences (95.0/87.6% similarity/identity) of the mouse and rat SULT1B1 sulfotransferases may indicate, in addition to their being orthologous enzymes, their functional importance in mammalian physiology. In addition to catalyzing the sulfation of Dopa and tyrosine isomers, the recombinant enzyme could also catalyze the sulfation of commonly used substrates, such as *p*-nitrophenol, dopamine and thyroid hormones, for other phenol sulfotransferase enzymes.

The functional relevance of the SULT1B1 sulfotransferases in the context of mammalian physiology remains to be clarified. However, in view of their unique substrate specificities, they can be hypothesized to be involved in catalysis of the sulfation of excess Dopa and tyrosine, thereby facilitating their removal from the body (*5-7*). This may be important physiologically since Dopa and tyrosine serve as precursors for the biosynthesis of catecholamines, that function as neurotransmitters or hormones *in vivo*. Excess Dopa and tyrosine, if not removed, may lead to overproduction of catecholamines and, consequently, abnormalities associated with the nervous and/or endocrine systems. As revealed in the tissue-specific expression experiment, the predominant presence of the mouse SULT1B1 sulfotransferase in the liver, an organ known to be involved in the removal of waste compounds, is in line with the hypothetical role mentioned above. A related issue regards the timing of the expression of the SULT1B1 sulfotransferase. Since this enzyme catalyzes the sulfation of catecholamine precursors, Dopa and tyrosine, it is possible that its expression may be correlated with the development of the nervous system and/or the endocrine system. Interestingly, the SULT1B1 sulfotransferase was found to be present at a very low level in liver samples from 1-day-old mice, and then gradually increased to the maximum level in liver samples from 4-week-old mice. These results provided the basis for further investigation of the molecular events involved in triggering of the expression of the SULT1B1 sulfotransferase gene during the developmental process. It will also be interesting to determine whether or not the onset of the SULT1B1 sulfotransferase gene expression is matched by the increase in the level of sulfated Dopa or tyrosine in the blood circulation. It should nevertheless be pointed out that, while postulating the hypothetical involvement of the SULT1B1 sulfotransferase in the homeostatic regulation of Dopa and tyrosine, activities of the enzyme toward other substrate compounds, *e.g.*, thyroid hormones, should still be kept in mind. As a cytosolic enzyme in liver cells, the SULT1B1 sulfotransferase, in concert with other known aryl sulfotransferases, is expected to exert its catalytic activity when it encounters the latter substrates.

In summary, we have in the present study cloned and sequenced a cDNA encoding a new mouse liver aryl sulfotransferase. Functional characterization of the recombinant enzyme expressed in COS-7 cells, combined with the sequence data, clearly indicated the identity of the newly discovered enzyme as SULT1B1 sulfotransferase. Northern blot analyses revealed the developmental stage-dependent and liver-specific expression of this new enzyme. More work is needed to clarify its functional involvement *in vivo*.

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