Characterization of rat iodothyronine sulfotransferases

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Departments of 1 Internal Medicine and 2 Pediatric Surgery, Erasmus Medical Center, 3015 GE Rotterdam, The Netherlands; 3 Department of Toxicology, German Institute of Human Nutrition, D-14558 Potsdam-Rehbrücke, Germany; and 4 Department of Molecular and Cellular Pathology, University of Dundee, Dundee DD1 8SY, Scotland, United Kingdom

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Kester, Monique H. A., Ellen Kaptein, Thirza J. Roest, Caren H. van Dijk, Dick Tibboel, Walter Meinl, Hansruedi Glatt, Michael W. H. Coughtrie, and Theo J. Visser. Characterization of rat iodothyronine sulfotransferases. Am J Physiol Endocrinol Metab 285: E592–E598, 2003.——Sulfation appears to be an important pathway for the reversible inactivation of thyroid hormone during fetal development. The rat is an often used animal model to study the regulation of fetal thyroid hormone status. The present study was done to determine which sulfotransferases (SULTs) are important for iodothyronine sulfation in the rat, using radioactive T4, T3, rT3, and 3,3′-T2 as substrates, 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as cofactor, and rat liver, kidney and brain cytosol, and recombinant rat SULT1A1, -1B1, -1C1, -1E1, -2A1, -2A2, and -2A3 as enzymes. Recombinant rat SULT1A1, -1E1, -2A1, -2A2, and -2A3 failed to catalyze iodothyronine sulfation. For all tissue SULTs and for rSULT1B1 and rSULT1C1, 3,3′-T2 was by far the preferred substrate. Apparent Km values for 3,3′-T2 amounted to 1.9 μM in male liver, 4.4 μM in female liver, 0.76 μM in male kidney, 0.23 μM in male brain, 7.7 μM for SULT1B1, and 0.62 μM for SULT1C1, whereas apparent Km values for PAPS showed less variation (2.0–6.9 μM). Sulfation of 3,3′-T2 was inhibited dose dependently by other iodothyronines, with similar structure-activity relationships for most enzymes except for the SULT activity in rat brain. The apparent Km values of 3,3′-T2 in liver cytosol were between those determined for SULT1B1 and -1C1, supporting the importance of these enzymes for the sulfation of iodothyronines in rat liver, with a greater contribution of SULT1C1 in male than in female rat liver. The results further suggest that rSULT1C1 also contributes to iodothyronine sulfation in rat kidney, whereas other, yet-unidentified forms appear more important for the sulfation of thyroid hormone in rat brain.

thyroid hormone; sulfation; rat sulfotransferase 1B1; rat sulfotransferase 1C1

Sulfation is a metabolic reaction that facilitates the excretion of endogenous and exogenous hydrophobic compounds in bile and urine by increasing their water solubility (5, 9, 16, 35). Biliary excretion of iodothyronines is also increased by sulfation. More importantly, however, sulfation appears to be a key step in the inactivation of thyroid hormone. The prohormone thyroxine (T4) is converted by outer-ring deiodination (ORD) to the biologically active 3,3′,5′-triiodothyronine (T3) or by inner-ring deiodination (IRD) to the inactive 3,3′,5′-triiodothyronine (rT3) (42). By sulfation, T3 loses its affinity for the thyroid hormone receptors (41). Additionally, T3 sulfate (T3S) is subject to accelerated degradation, as sulfation facilitates the IRD of T3 by the type I deiodinase (D1) (42). Sulfation also facilitates the inactivating IRD of T4 by D1, whereas the activating ORD of T4 by D1 is completely blocked by sulfation (42). Therefore, an important function of sulfation is to facilitate the irreversible degradation of thyroid hormone. Furthermore, under conditions in which the deiodinative clearance of sulfates is impaired, sulfation may be reversed by sulfatases. Because T3S and T4S levels in the human fetal circulation are high (4, 38, 40), it has been speculated that sulfation is a mechanism to protect the fetus from excessive T3 and that sulfation/desulfation plays an important role in the regulation of thyroid hormone bioactivity during fetal development (27, 34, 39). The exact mechanism for the increased iodothyronine sulfate levels in the fetal circulation is unclear, but the reversible nature of this inactivation step contrasts with the irreversible nature of type III deiodinase (D3)-catalyzed IRD, which is also extensive during fetal development (3, 14, 15, 26, 33).

Sulfation is catalyzed by cytosolic sulfotransferases present in a wide range of tissues. The sulfotransferases transfer the sulfuryl group of 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to usually OH groups of their substrates (5, 16). All cytosolic sulfotransferases are members of a single gene superfamily, termed SULT. A systematic nomenclature is in preparation but not yet finalized. It is already widely used for human (h)SULTs but not for rat (r)SULTs. Table 1 indicates the designations of the rSULTs used in the present study together with synonymous names that have been used elsewhere. On the basis of amino acid sequence homology, three families of sulfotransferases

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RAT IODOTHYRONINE SULFOTRANSFERASES

Table 1. Designation of rat sulfotransferases

<table>
<thead>
<tr>
<th>Designation Used in This Publication</th>
<th>Designation Used in Other Publications</th>
<th>GenBank Accession</th>
<th>No. of Amino Acids</th>
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<tr>
<td>SULT1A1</td>
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<td>SULT1B1</td>
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</tr>
<tr>
<td>SULT1C1</td>
<td>ST1C1, HAST-1</td>
<td>A49098</td>
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<td></td>
<td>S96131P</td>
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<td>SULT1C3</td>
<td>ST1C3A</td>
<td>CAB41461</td>
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<td>AAC99890</td>
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</tr>
<tr>
<td>SULT1E1</td>
<td>ST1E2, RST-1, RST-3</td>
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<td>295</td>
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</tr>
<tr>
<td>SULT2A1</td>
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<td>A34822</td>
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</tr>
<tr>
<td>SULT2A2</td>
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</tr>
<tr>
<td>SULT2A3</td>
<td>ST2A5, ST-60</td>
<td>BAA03634</td>
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</tr>
<tr>
<td>SULT4A1</td>
<td>rBR-STL</td>
<td>AAFF61198</td>
<td>284</td>
</tr>
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</table>

1 Not investigated in the present study. 2 The cDNA-deduced amino acid sequence differs from A49098 in three residues (S2A, T60A, S96P).

have been identified in humans: the SULT1 family, which primarily represents phenol sulfotransferases, including hSULT1A1, -1A2, -1A3, -1B1, -1C2, -1C4, and -1E1, the SULT2 family, which usually prefers alcoholic substrates, including hydroxysteroids, and the SULT4 family, containing sulfotransferase-like proteins for which no substrates have been identified yet (5, 9, 16, 35). In the rat, the phenol sulfotransferases rSULT1A1, -1B1, -1C1, -1C2, -1C3, -1D1, -1E1, and -1E2, the hydroxysteroid sulfotransferases rSULT2A1, -2A2, and -2A3, and the sulfotransferase-like protein rSULT4A1 have been cloned (5, 9, 16, 18, 35, 45). For several human and rat phenol sulfotransferases, allelic variants have been identified (5, 9, 16, 18, 45). Another important observation is that the sulfotransferases may not exist only as homodimers but also as heterodimers (25).

Sulfation of iodothyronines is catalyzed by phenol sulfotransferases. Recently, we identified hSULT1A1, -1A3, -1B1, and 1E1 as human iodothyronine sulfotransferases (23). For several human and rat phenol sulfotransferases, allelic variants have been identified (5, 9, 16, 35). The enzyme preparations. rSULT1A1, -1E1, -2A1, -2A2, and -2A3 did not catalyze iodothyronine sulfation (data not shown).

Materials. Male and female Wistar rat liver cytosols and male Wistar rat kidney and brain cytosols were obtained as previously described (43). Approval was obtained from the Erasmus Committee of Animal Welfare. rSULT1C1 cDNA (32) was kindly provided by Dr. Y. Yamazoe and expressed in V79 cells as previously described (17). rSULT1A1 cDNA (20) was kindly provided by Dr. C. N. Falany and expressed in Salmonella typhimurium (18). rSULT2A1, -2A2, and -2A3 were cloned and expressed in S. typhimurium, and rSULT2A1 was also expressed in V79 cells (6, 17). rSULT1B1, an rSULT1C1 variant containing amino acid substitutions S2A, T60A, and S96P, and rSULT1E1 and -2A3 were cloned by RT-PCR and expressed in S. typhimurium (18). V79 and bacterial cell cytosols were prepared as previously described (17).

Results

Figure 1 shows the sulfation of 0.1 μM T4, T3, rT3, and 3,3′-T2 by male and female rat liver cytosol, male rat kidney, and brain cytosol, and rSULT1B1 and -1C1 in the presence of 50 μM PAPS. All enzyme preparations show a substrate preference for 3,3′-T2. Rates of 3,3′-T2 sulfation are >50-fold higher than those of T3 and rT3 sulfation; T4 is the poorest substrate for all enzyme preparations. rSULT1A1, -1E1, -2A1, -2A2, and -2A3 did not catalyze iodothyronine sulfation (data not shown).

Figure 2 shows the sulfation of 3,3′-T2 by female rat liver or male rat liver, kidney, or brain cytosol as a function of the substrate concentration. Maximum sulfation rates were obtained at ~10 μM 3,3′-T2 in male and female rat liver cytosol, at ~2 μM in male rat kidney cytosol, and at ~1 μM in male rat brain cytosol. Rat brain cytosol showed clear substrate inhibition for 3,3′-T2 at concentrations above 1 μM. Km and Vmax values for the different tissue cytosols were calculated.
from the linear double-reciprocal plots of sulfation rate vs. 3,3'-T2 concentration and are presented in Table 2. V_max values decreased in the order male liver > female liver > brain > kidney. K_m values for T3 sulfation by the tissue cytosols, which were determined under the same conditions, were >50-fold higher than for the sulfation of 3,3'-T2 (data not shown).

Figure 3 depicts the sulfation of 3,3'-T2 by rSULT1B1 or -1C1 as a function of the substrate concentration. For rSULT1C1, maximum sulfation rates were obtained at lower 3,3'-T2 concentrations than for

Table 2. Kinetic parameters of rat iodothyronine sulfotransferases

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>K_m, μM</th>
<th>V_max, pmol·min⁻¹·mg protein⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate: 3,3'-T2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male liver cytosol</td>
<td>1.85 ± 0.45</td>
<td>2,042 ± 400</td>
</tr>
<tr>
<td>Female liver cytosol</td>
<td>4.35 ± 0.59</td>
<td>1,516 ± 214</td>
</tr>
<tr>
<td>Kidney cytosol</td>
<td>0.76 ± 0.05</td>
<td>15.6 ± 1.9</td>
</tr>
<tr>
<td>Brain cytosol</td>
<td>0.23 ± 0.01</td>
<td>32.0 ± 0.3</td>
</tr>
<tr>
<td>rSULT1B1 (Salmonella)</td>
<td>7.74 ± 1.46</td>
<td>6,029 ± 1,146</td>
</tr>
<tr>
<td>rSULT1C1 (V79 cells)</td>
<td>0.62 ± 0.16</td>
<td>251 ± 93</td>
</tr>
<tr>
<td>Substrate: T3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rSULT1B1 (Salmonella)</td>
<td>142 ± 9</td>
<td>1,156 ± 133</td>
</tr>
<tr>
<td>rSULT1C1 (V79 cells)</td>
<td>100 ± 6</td>
<td>50.8 ± 6.3</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD of 2–6 experiments. Incubations were done with 50 μM 3'-phosphoadenosine-5'-phosphosulfate. T3, diiodothyronine; T2, 3,3',5'-triiodothyronine.

Fig. 1. Sulfation of iodothyronines by male (M) and female (F) rat liver cytosol, male rat kidney, and brain cytosol, rat sulfotransferase (rSULT)1B1 and rSULT1C1. Reaction conditions were 0.1 μM [3,3'-125I]T2, 3,3',5-triiodothyronine (T3), inactive 3,3',5'-T3 (rT3), or 3,3'-diiodothyronine (T2), 0.1 mg protein/ml, 50 μM 3'-phosphoadenosine-5'-phosphosulfate (PAPS), and 30-min incubation. Results are means of triplicate determinations from a representative experiment.

Fig. 2. Effects of substrate concentration on sulfation of 3,3'-T2 by female or male rat liver cytosol, male rat kidney, or brain cytosol. Insets: double-reciprocal plot. Reaction conditions were 0.1–25 μM [3,3'-125I]T2, 25 (male liver), 50 (female liver and male brain) or 250 (male kidney) μg protein/ml, 50 μM PAPS, and 30-min incubation. Results are means of triplicate determinations from a representative experiment.

Fig. 3. Sulfation of 3,3'-T2 by rSULT1B1 or -1C1 as a function of the substrate concentration. For rSULT1C1, maximum sulfation rates were obtained at lower 3,3'-T2 concentrations than for
The decrease in sulfation rate for rSULT1B1 at concentrations above 1 μM indicated substrate inhibition. The apparent $K_m$ values calculated from the Lineweaver-Burk plots amounted to 7.7 μM for rSULT1B1 and 0.62 μM for rSULT1C1 (Table 2). Because crude cytosols of rSULT1B1-expressing Salmonella cells and rSULT1C1-expressing V79 cells were tested, the $V_{\text{max}}$ values for the different enzymes are not representative of their $K_{\text{cat}}$ values. The kinetic parameters for T3 sulfation by the different isoenzymes are also presented in Table 2. Compared with 3,3′-T2, apparent $K_m$ values for T3 were 20- to 150-fold higher. The apparent $K_m$ value determined for 3,3′-T2 sulfation by the rSULT1C1 variant (5.8 μM) was 10-fold higher than for wild-type rSULT1C1.

Because of its higher affinity for the different sulfotransferases, 3,3′-T2 was used in different experiments as a model substrate for the receptor-active T3. Figure 4 depicts the sulfation of 1 μM 3,3′-T2 by male rat liver cytosol at different PAPS concentrations (1–100 μM). Maximum sulfation rates were reached at PAPS concentrations ≥30 μM. Its apparent $K_m$ value, calculated from the Lineweaver-Burk plot, was 4.7 μM. The $K_m$ values for the other enzyme preparations were also in the low micromolar range, i.e., 3.8 μM for female rat liver, 2.2 μM for male rat kidney, 3.5 μM for brain cytosol, 2.0 μM for rSULT1B1, and 6.9 μM for rSULT1C1.

Figure 5 shows the effects of increasing concentrations (1–100 μM) of unlabeled iodothyronines on the sulfation of [3,3′-125I]T2 by male rat liver cytosol. 3,5-T2 had no effect; all other iodothyronines inhibited the sulfation of labeled 3,3′-T2 dose dependently, in the order 3,3′-T2 > 3′-T1 > 3′,5′-T2 > rT3 > T4 > T0 > 3-T1 > T3.

Figure 6 compares the effects of 10 μM unlabeled iodothyronines on the sulfation of 1 μM [3,3′-125I]T2 by male and female liver and male kidney and brain cytosol, rSULT1B1, and rSULT1C1. 3,3′-T2 sulfation
by rSULT1C1 was affected most by the different iodothyronines. Sulfation of 3,3'-T2 by female rat liver cytosol was inhibited less potently by the different analogs than 3,3'-T2 sulfation by male rat liver. The structure-activity relationships for inhibition of T2 sulfation by analogs were similar for female and male liver, kidney, rSULT1B1, and rSULT1C1. In general, iodothyronines without iodine substituent in the outer ring (T0, 3-T1, 3,5-T2) and those with two iodines in the inner ring (3,5-T2, T3, T4) showed little or no inhibition. In other words, iodothyronines that showed significant inhibition had zero or one iodine substituent in the inner ring and one or two iodines in the outer ring.

The inhibition profiles for rat liver and kidney were significantly correlated with those for SULT1B1 and -1C1, with coefficients varying between 0.869 and 0.990. However, in contrast to all other enzyme preparations, 3,3'-T2 sulfation by rat brain cytosol was poorly inhibited by 3'-T1 and 3',5'-T2, and the inhibition profile for rat brain cytosol also showed weaker correlations with those for rSULT1B1 (r = 0.814) and rSULT1C1 (r = 0.633).

**DISCUSSION**

In previous studies, hSULT1A1, -1A2, -1A3, -1B1, -1C1, and -1E1 have been identified as important enzymes for iodothyronine sulfation in humans (1, 13, 23, 28, 29, 47). rSULT1A1, -1B1, -1C1, and -1E1 show 79, 74, 63, and 70% amino acid sequence identity, respectively, with their human homologs, and ~50% identity among themselves. Sulfation of T3 by rat SULT1B1 and -1C1 has been reported previously (12, 19, 37, 44). In this study, we compared kinetic parameters and substrate specificities for the different rat enzymes with these characteristics for female rat liver and male rat liver, kidney, and brain cytosol in an attempt to determine which enzyme forms are involved in iodothyronine sulfation in the different tissues. We used mammalian V79 cells and bacterial *S. typhimurium* cells as expression systems for the different SULT enzymes. Previous studies showed that the different systems give similar results for the various human SULT enzymes (23).

Iodothyronine sulfotransferase activities in rat liver and kidney and of rSULT1B1 and -1C1 showed very similar substrate specificities. The higher maximum sulfation rates observed in male than in female rat liver cytosol are in agreement with earlier reports on the sex dependence of T3 sulfation in rats, which is explained by the male-dominant expression of rSULT1C1 (21, 22, 30, 36). rSULT1C1 is predominantly expressed in male liver, kidney, and intestine, whereas rSULT1B1 expression in liver, kidney, and intestine is equal in male and female rats (2, 7, 8). The apparent $K_m$ of 3,3'-T2 in liver cytosol is between the $K_m$ values for SULT1B1 and -1C1, in male liver closer to that for SULT1C1, and in female liver closer to that for SULT1B1, supporting a greater contribution of SULT1C1 in male vs. female rat liver. The apparent $K_m$ of 3,3'-T2 in kidney is similar to the $K_m$ for rSULT1C1, suggesting that SULT1C1 is a more important enzyme than SULT1B1 in rat kidney. Although direct comparison between rSULT1B1 and rSULT1C1 mRNA levels in the different tissues is difficult, Dunn and Klaassen (8) showed that rSULT1C1 mRNA expression is >100-fold higher in liver than in kidney, whereas rSULT1B1 mRNA levels are 10-fold higher in liver than in kidney. In agreement with this, much higher sulfation rates were found in liver than in kidney cytosols.

It is, however, possible that, besides SULT1B1 and -1C1, SULT1C2, -1C3, and -1D1 also contribute to
iodothyronine sulfation in the different tissues. Furthermore, rat phenol sulfotransferases have been demonstrated to exist not only as homodimers but also as heterodimers (25). Thus, besides SULT1A1, -1B1, and -1C1 homodimers, tissues such as liver may contain various heterodimers. Although SULT1A1 homodimer does not possess sulfotransferase activity toward iodothyronines, it is not excluded that SULT1A1/1B1 and -1A1/1C1 heterodimers catalyze iodothyronine sulfation. It is clear that substrate specificities and apparent $K_m$ values determined in tissue represent average values for mixtures of homo- and heterodimeric iodothyronine sulfotransferases. Substrate preference and $K_m$ values of 3,3'-T$_2$ in rat brain are different from those of SULT1B1 and -1C1. Therefore, other enzyme forms(s) seem to be involved in iodothyronine sulfation in rat brain. In agreement with this, no rSULT1B1 and rSULT1C1 mRNAs were detected in rat brain (8). A possible candidate is the recently cloned rat brain sulfotransferase-like protein rSULT4A1 (11). Compared with liver and kidney, the inhibition profile for rat brain cytosol showed weaker correlations with those for SULT1B1 and -1C1, also indicating the involvement of other enzymes. However, assessment of inhibition profiles may be biased if inhibitors are extensively sulfated themselves by the enzymes under study or other sulfotransferases, resulting in a decrease in their inhibitory potency. For instance, the weaker inhibition of 3'-T$_1$ in rat brain may be explained by its sulfation by different enzymes present in brain.

Concerning the rSULT1C1 variant, mutational analysis should reveal which amino acid substitution (S2A, T60A, or S69P) contributes most to the 10-fold lower affinity of the rSULT1C1 variant enzyme for 3,3'-T$_2$ compared with the wild-type rSULT1C1. Previous studies showed that hSULT1A1 efficiently sulfates iodothyronines, whereas the rSULT1A1 homolog does not catalyze iodothyronine sulfation (44). The human estrogen sulfotransferase hSULT1E1 also efficiently catalyzes iodothyronine sulfation (24). However, because estrone and estradiol are inefficient substrates for the rat homolog rSULT1E1 (10), it is not surprising that no catalytic activity toward iodothyronines was detected for this enzyme. Still, iodothyronine sulfation by rSULT1E2 is not excluded. In rats as well as in humans (Kester MHA, Coughtrie MWH, Glatt H, and Visser TJ, unpublished observations), hydroxysteroid sulfotransferases do not appear to contribute importantly to iodothyronine sulfation.

Because all enzymes prefer 3,3'-T$_2$ as substrate, in this study we have used 3,3'-T$_2$ as the model substrate for the receptor-active T$_3$. Physiologically, T$_3$ is perhaps the most important substrate, since sulfation is an important pathway for the inactivation of the hormone. However, a physiological role for 3,3'-T$_2$ cannot be excluded. The diiodothyronines 3,3'-T$_2$ and 3,5-T$_2$ have been shown to stimulate mitochondrial thermogenesis by direct mitochondrial binding (31a). Furthermore, Wu et al. (46) showed that, in late gestation in sheep, 3,3'-T$_2$ sulfate is the most abundant iodothyronine metabolite transferred from the fetus to the mother. Possibly, sulfation of 3,3'-T$_2$ and the transfer of 3,3'-T$_2$ sulfate from fetus to mother protects the fetus from excessive mitochondrial respiration.

In conclusion, rSULT1B1 and -1C1 appear to be important enzyme forms for sulfation of iodothyronines in rat liver and kidney, with proportionally greater contributions in kidney than in liver and in male than in female liver. Other, still unidentified enzymes appear to be responsible for iodothyronine sulfation in rat brain. Further studies are needed to determine the role of these sulfotransferases in the regulation of (fetal) thyroid hormone status.

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DISCLOSURES

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