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Iodothyronine Sulfotransferase Activity in Rat Uterus During Gestation

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ABSTRACT

In developing mammals, we and others demonstrated that sulfation is an important pathway in the metabolism of thyroid hormone, and there is significant fetal-maternal transfer of sulfated iodothyronine. In the present study, we characterized a novel iodothyronine sulfotransferase (IST) in pregnant rat uterus. ^{125}I-labeled 3,3'-diiodothyronine (T_2), T_3, rT_3, and T_4 were used as substrates with unlabeled 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as the sulfate donor. Sulfated iodothyronine products were separated by Sephadex LH-20 column and further identified on reverse phase HPLC. We measured IST activity in pregnant rat uterus by incubating 1 μM substrate, 50 μM PAPS, and 50 μg cytosol protein, pH 7.2, 30 min at 37°C. The results show that the substrate preference of the uterine IST activity is: $T_2 > rT_3 > T_3 > T_4$; the pH optimum is 6.0 for T_2. The K_m and V_{max} (for gestational day 21 uterus) for T_2 are 0.62 μM and 3466 pmol/mg protein/h, respectively; for PAPS the values are 2.6 μM and 1523 pmol/mg protein/h, respectively. During pregnancy, the total activities exhibit a U-shaped curve with minimum activity at day 13 of gestation; while a thermostable activity increases significantly near term. In summary, there is significant uterine IST that varies during pregnancy. The role of this uterine sulfotransferase activities in regulating the bioavailability of thyroid hormone in the developing fetus remains to be elucidated. **(*Pediatr Res* 48: 847–851, 2000)**

ABBREVIATIONS

IST, iodothyronine sulfotransferase
T_2, 3,3'-diiodothyronine
T_3, 3,3',5-triiodothyronine
rT_3, reverse T_3
T_4, thyroxine
PAPS, 3'-phosphoadenosine-5'-phosphosulfate
D3, type III deiodinase activity
NP, nonpregnant

In developing mammals, deficiency or excess of thyroid hormones during the fetal and neonatal periods can lead to morphologic and functional abnormalities of the CNS (1–7). Three types of monodeiodinases, type 1, 2, and 3, have been identified in mammalian fetal tissues and are thought to play roles in the regulation of active hormone (T_3) generation in different tissues and organs which have specific temporal patterns of development (8, 9). Additionally, in ovine fetuses, we have shown that alternate sulfate pathways are major routes of thyroid hormone metabolism and that high concentrations of thyroid hormone sulfoconjugates, *i.e.* thyroxine (T_4S) and 3,3',5-triiodothyronine (T_3S) and their metabolites (3,3',5'-triiodothyronine, rT_3S, and 3,3'-diiodothyronine, T_2S), are present in the biologic fluids of human and ovine fetuses (10–18). More recently, we detected significant levels of sulfated iodothyronines in the plasma of premetamorphic tadpoles before there are detectable amounts of nonconjugated thyroid hormones (19). Sulfoconjugation may accelerate further degradation and excretion of the thyroid hormone and may provide a reservoir for biologically active hormones such as T_3 (20–22) and possibly rT_3 and T_2 (23, 24), which can be recovered from sulfated iodothyronines by sulfatases in selective tissue where hormone action is required. In addition, by accelerating further deiodination, this could assist in recovering iodine for new hormone synthesis.

This sulfoconjugation may be linked with monodeiodination in regulating bioavailability of thyroid hormones in various tissues. Thyroid hormones are small diffusible lipophilic compounds that may be able to reach the fetus through the surrounding mammalian uterus. Recently, Galton *et al.* found high levels of type 3 monodeiodoinase activity in pregnant rat

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uterus, which suggests a possible role for this tissue in the regulation of the amount of maternal thyroid hormone that reaches the fetus (25). We hypothesize that iodothyronine sulfotransferase (IST) may be present in rat uterus and contribute significantly to regulation of the bioavailability of thyroid hormones to the fetus. In the present study, we characterized IST activity in the pregnant rat uterus at various gestational ages.

MATERIALS AND METHODS

Animals. Timed-pregnant rats (12–14 wk old) were purchased from the Charles River Laboratory (North Wilmington, MA, U.S.A.). Rats were housed under conditions of controlled lighting and temperature until killed for study between gestational day (d)9 and d21. Virgin female rats of comparable age were purchased from the same vendor to provide control data in the nonpregnant uterus. All animal protocols were approved by the Animal Use Subcommittee at the VA Medical Center, Long Beach, CA, U.S.A.

Sulfotransferase assay. Rats were killed by decapitation under anesthesia by intraperitoneal infusion of ketamine (20 μg/kg). The uterus was rapidly removed and dissected free of surrounding adipose tissue. It was then cross-sectioned to separate the individual implantation site. At d9 and d11 no further dissection was performed. At d13 to d21, the implantation site was opened by making a longitudinal cut along the antimesometrial side of the uterine wall. This part of the uterus lies directly on the dorsal side of the fetus and is on the opposite side of the attachment of the placenta to the fetus. The uterus was folded back over the amniotic sac containing the fetus and the placenta, and was then gently peeled free from these tissues. The amniotic sac, fetus, and placenta were then separated.

Uterine tissue was homogenized in 0.25 M sucrose, 10 mM HEPES, and 1 mM DTT (pH 7.0) and the cytosol was prepared by centrifuging at 105,000 $\times$ *g* for 1h. Protein was measured by a modified Lowry Method (26). Sulfotransferase activities were assayed in duplicates using the modified method of Kaptein and coworkers (20). In brief, 1 μM T_2 (or T_3, rT_3, or T_4) was incubated at 37°C for 30 min with 100,000 cpm of the ^{125}I-labeled compound in the presence or absence (blank) of 50 μM PAPS in 0.2 mL 0f 0.1 M phosphate (pH 7.2) and 2 mM EDTA (assay buffer). The reactions were started by addition of cytosol diluted in ice-cold assay buffer and stopped by addition of 0.8 mL 0.1 N HCl. The mixtures were applied to a Sephadex LH-20 minicolumn (bed volume 1 mL) equilibrated with 2 volumes of 0.1 N HCl. Iodide, sulfated iodothyronines, and nonsulfated iodothyronines were successively eluted with 3 $\times$ 1 mL 0.1 N HCl, 8 $\times$ 1 mL ethanol:water (20:80, vol:vol), and 4 $\times$ 1 mL ethanol/0.1 N NaOH (50:50, vol:vol), respectively. One- milliliter fractions were collected and counted for radioactivity. Sulfation in complete reaction mixtures was corrected by subtracting PAPS blank. The results are the means of 2–3 separate experiments and are specified in the legends of figures. Sulfated iodothyronines were further identified by high-pressure liquid chromatography (μBondapak C_{18} column) isocratically with a mixture of acetonitrile and 0.02 M ammonium acetate, pH 4.0 (22:78, vol:vol) as described previously (22).

Sources of materials. 3,3'-T_2, T_3, 3,3',5'-T_3 (rT_3) and T_4 were purchased from Henning-Berlin (Berlin, Germany) [3'-^{125}I]. - rT_3, -T_4, -T_3, and -T_2 were prepared by radioiodination using the method previously described (27). PAPS, DTT and Sephadex LH-20 were purchased from Sigma Chemical Co. Chemical (St. Louis, MO, U.S.A.)

Statistical analysis. Student's unpaired *t* test was used to compare differences between groups. Analysis of variance was used to test the multigroup comparisons. If significant differences were present, Dunnett's multicomparison test was used to compare the control or baseline mean and the mean values of other groups (28). Significance was defined as $p < 0.05$. Results are reported as means $\pm$ SE.

RESULTS

Characterization of rat uterine iodothyronine sulfotransferase activity. In preliminary experiments, sulfation of labeled iodothyronines, T_2, rT_3, T_3, and T_4 increased linearly with increasing concentrations of cytosol protein at 30 min incubation. At optimal enzyme concentrations, the production of sulfated iodothyronines is linear with time up to 1h (results not shown). We further found no significant deiodination in the uterine cytosol preparations when incubated up to 3h with labeled iodothyronines. Figures 1 and 2 compare the effect of increasing concentrations of unlabeled T_2, rT_3, T_3, and T_4 on the sulfation of radioactive T_2 and rT_3, respectively, by rat d21 pregnant uterine cytosol in the presence of PAPS. The dose inhibition curves for unlabeled rT_3 and T_2 were very similar when their effects on sulfation of radioactive T_2 and rT_3 were compared. In both cases IC_{50} values were nearly 1.5 times

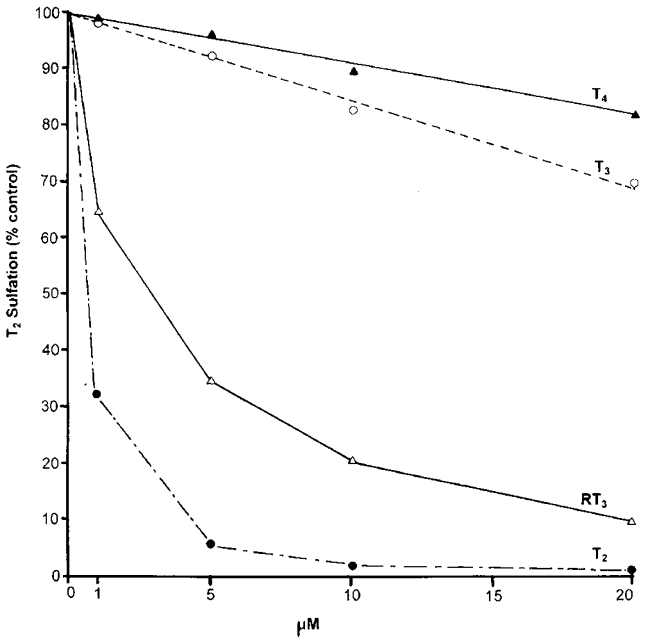

Figure 1. Effect of 1–20 μM unlabeled 3,3'-T_2, rT_3, T_3 and T_4 on the sulfation of 3,[3'-^{125}I]T_2 by rat d21 pregnant uterine cytosol in the presence of 50 μM PAPS and 30 min incubation at 37°C. Results are the means of two separate experiments.

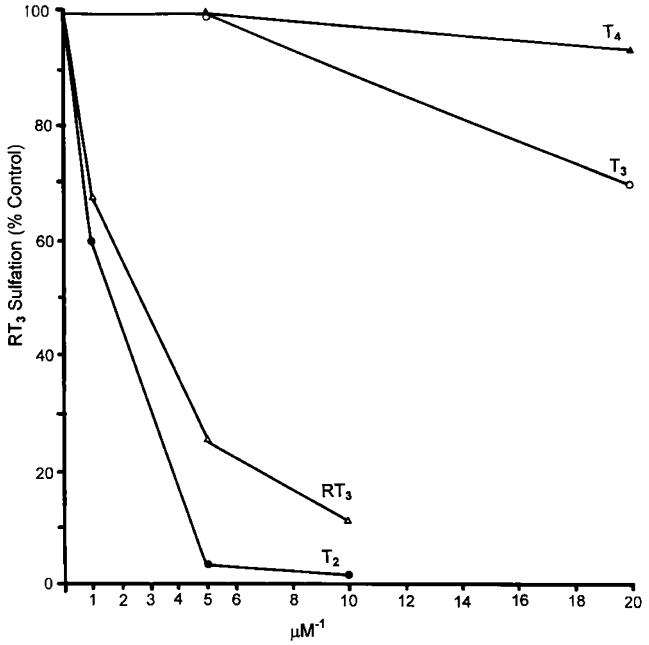

Figure 2. Effect of 1–20 μM unlabeled T_3 and T_4 and1–10 μM unlabeled 3,3'-T_2 and rT_3 on the sulfation of 3,5', [3'-^{125}I]T_3 by rat 21d pregnant uterine cytosol in the presence of 50 μM PAPS, 250 μg protein/mL and 30 min incubation at 37°C. The means of two separate experiments are shown.

higher for rT_3 than for T_2. These results suggest T_2 and rT_3 (and possibly T_3) are substrates for sulfotransferase isoenzyme(s) that is (are) more readily saturated by T_2, and rT_3 than by T_3.

Figure 3 shows the effects of pH on the sulfation of T_2 by uterine tissue from virgin (NP) and d21 pregnant rat. In both preparations, peak T_2 sulfation rates were observed at pH 6.0. However, all subsequent experiments were performed at a more physiologic pH of 7.2.

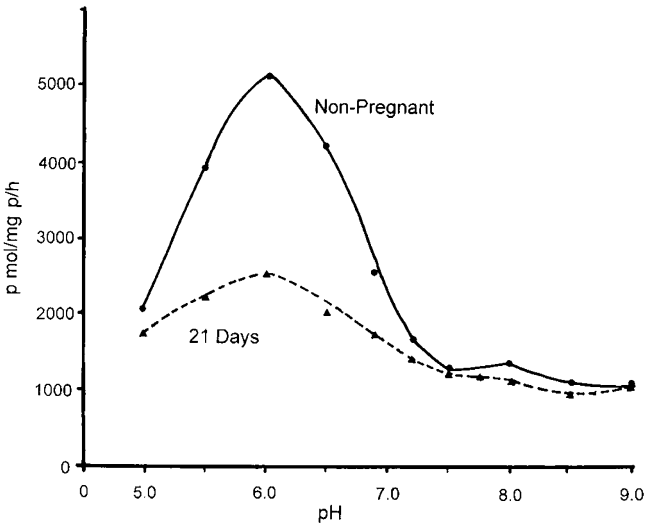

Figure 3. Effect of pH on sulfation of 3,3'-T_2 by nonpregnant (NP) and d21 pregnant uterine cytosol in 1 μM 3,[3'-^{125}I]T_2, 250 μg cytosolic protein/mL, 50 μM PAPS and 30 min incubation. Results are the means of 2–3 separate experiments.

The double reciprocal plots of sulfation rates (in pmol/mg protein/h) *versus* T_2 concentration (μM) were linear allowing the calculation of apparent K_m for T_2 and V_{max} (at 50 μM PAPS). Table 1 presents the kinetic data (n = 3–5) showing that the apparent K_m value for T_2 for d21 was similar to uterine cytosol from NP, d8, d10, and d19.

The double reciprocal plots of increasing PAPS concentration (0.5–10 μM) on the sulfation of 1 μM T_2 by NP, and pregnant uterine cytosol were linear. In all preparations, T_2 sulfation approached maximum rates at PAPS concentration near 10 μM. The kinetic data from 3–4 such experiments in each gestational period are shown in Table 1, showing that the apparent K_m value for PAPS is slightly higher in NP than in d21 uterus; but there is no significant statistical difference.

From Figure 4 an apparent K_m value of 3.7 μM and V_{max} of 77 pmol/mg p/h were calculated for T_3 sulfation by uterine cytosol from NP rats in the presence of 50 μM PAPS. Therefore, the apparent K_m value for T_3 is higher and the V_{max} value is much lower than the corresponding values for T_2 sulfation.

Gestational changes of iodothyronine sulfotransferase activity and their thermostability. In Figure 5, the IST activities in uterus tissue from the pregnant rats are plotted *versus* gestation age. The activities show a U-shaped curve with a minimum activity at d13 and higher values at d8 and d21. There was also significant IST activities in the uterine tissue from nonpregnant (NP) rats. However, when heated to 45°C for 15 min, only about 4.2% of T_2-ST remained in NP, while 67.0% ($p < 0.01$ cf. NP) was stable in the d21 pregnant uterus. The thermostable IST activity increased significantly near term at d21.

DISCUSSION

The present studies demonstrate the presence of IST activity in rat uterine cytosol. The enzyme activity has a substrate preference for T_2 ($T_2 > rT_3 > T_3 > T_4$) and a pH-optimum of 6.0. The most interesting finding is that the activity appears to be gestation age dependent, especially the thermostable (45°C × 5 min) form, which increase significantly near term. The uterine sulfotransferase activity is clearly quite different from

Table 1. *Gestational Changes of Kinetic Parameters of T_2 Sulfation by Rat Uterine Cytosol**

		N	K_m	V_{max}
*3,3'-T_2***	NP	3	0.58 ± 0.06	2733 ± 120
	d8	3	1.05 ± 0.05	2600 ± 200
	d10	3	0.50 ± 0.08	1065 ± 235
	d13	3	1.53 ± 0.21	565 ± 115
	d19	3	1.00 ± 0.23	1310 ± 690
	d21	5	0.62 ± 0.18	3466 ± 1438
PAPS	NP	4	3.3 ± 0.3	1507 ± 59
	d8	3	2.9 ± 0.3	730 ± 45
	d10	3	3.7 ± 1.2	583 ± 94
	d13	—	—	—
	d19	3	2.6 ± 0.3	363 ± 37
	d21	3	2.6 ± 0.5	1523 ± 442

Data are presented as mean ± SE μM (K_m) or pmoles T_2S/mg prot./h. (V_{max}) with number of experiments (N) indicated.

* Values are calculated from double-reciprocal plots; ** Determined at 50 μM PAPS

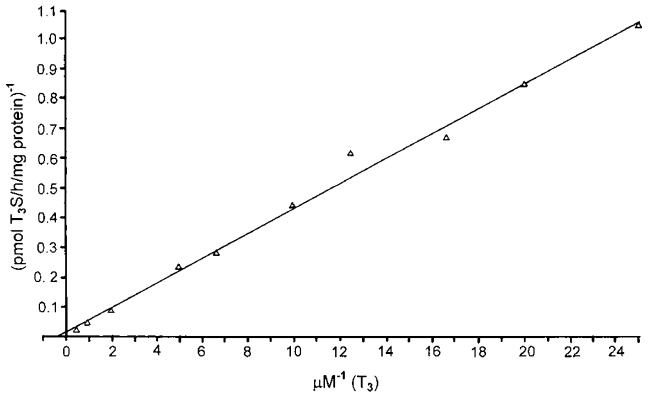

Figure 4. Double-reciprocal plot of the effect of substrate concentration on the sulfation of T_3 by a NP rat uterine cytosol. Reactions were conducted in duplicated in 0.04–2 μM 3,5,[3'-^{125}I]-T_3, 50 μM PAPS, 1 mg cytosol protein for 60 min at 37°C. Results are the means of 2–3 separate experiments.

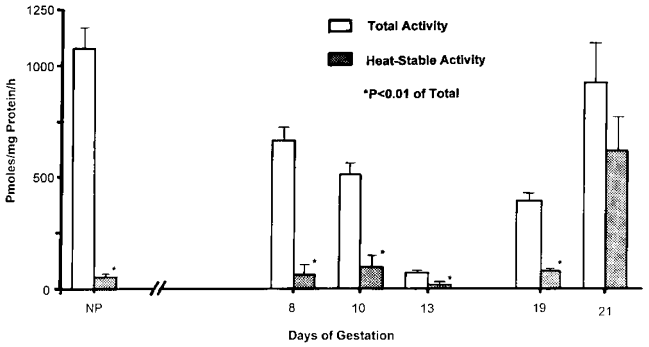

Figure 5. Effect of gestational changes on sulfation of 3,3'-T_2 by pregnant uterus cytosol: from NP to d21 gestation. The open bar represents total activities and the shaded bar represents thermostable activity after heating up at 45°C for 5 min. Reactions (n = 3–5 separate experiments) were conducted in 1 μM 3,[3'-^{125}I]T_2, 250 μg protein/mL, 50 μM PAPS and 30 min incubation.

rat hepatic isozyme (rSULT1C1), which has a pH-optimum of 8.0 and a substrate preference of $T_2 > T_3 > rT_3 > T_4$ (21). In addition, the apparent Michaelis-Menton (K_m) value for T_2 is lower (0.50–1.53 μM) in uterus than in liver (4.9 μM for female rats). At 1 μM T_2 and pH 7.2, the apparent K_m values for PAPS were similar in uterus and liver [2.6–3.7 μM and 3.8 μM (for female rats), respectively]. Kester *et al.* reported that human estrogen sulfotransferase (SULT1E1), which is expressed in the uterus and possibly also in the placenta, is also found to sulfoconjugate iodothyronines (29). However, SULT1E1 has a much higher K_m to T_2 (3.5–6.0 μM) and to T_3 (15.3–36.1 μM) than the cytosol enzyme in rat uteri in the present study. Presently, one cannot exclude the possibility that a similar rat SULT1E1 may conjugate both estrogen and thyroid hormone as shown in the human. In addition, the uterine IST is quite different from human SULT1A1 and SULT1A3 (30). Although the pH optimum is similar to the phenolsulfotransferase (EC 2.8.2.1) described in rat brains, sulfation of iodothyronines was not measured in that study (31).

Recently, Galton *et al.* found high levels of type III deiodinase activity (D3) in pregnant rat uterus (25). Furthermore, by *in situ* hybridization, both D3 mRNA and activity were present at the implantation site as early as gestational age d9 before placental development. Notably, the levels of D3 in the pregnant uterus are even higher than those in the placenta, which were determined at the same stage of gestation, and are much higher than those in either the whole fetus or in the individual fetal organs. At later stages of gestation, uterine D3 activities remains very high (25). These findings suggest that pregnant uterus plays an important role in modulating fetal exposure to maternal thyroid hormone. The present studies demonstrate significant levels of sulfotransferase activities, using D3 metabolites (T_2 and rT_3) as preferred substrates, in the pregnant rat uterus. This appears to suggest a potential role for the sulfation pathway in regulating, together with uterine and placental D3, the amount of maternal thyroid hormones that reach the fetus. It is interesting that the uterine sulfotransferase activities may be under gestational regulation with a minimum level of both thermostable and thermolabile activities around d13 that coincides with localization of expression of D3 in the epithelial cells lining the uterine lumen that surrounds the fetal cavity (25). This is consistent with the hypothesis that uterine IST is an added mechanism linked with D3 to optimize maternal to fetal transfer of thyroid hormone in the developing fetus before and after the onset of fetal thyroid function (d18–19) (25, 32).

The sulfoconjugation of thyroid hormones (T_4 and T_3) and their metabolites (rT_3 and T_2) may accelerate further degradation and excretion of iodothyronines. Sulfated iodothyronine may also serve as a reservoir for biologically active hormones such as T_3, which can be recovered from T_3S by sulfatases in selective tissue in which hormone action is required (21). Sulfated T_3 may be metabolized to active hormone by tissue sulfatase particularly in situations when type 1 deiodinating activity is low, *e.g.* fetal state and nonthyroidal illnesses (33, 34). The purpose of rapid sulfation of 3,3'-T_2 and rT_3 by uterine IST is unknown. Both metabolites have little affinity for the nuclear thyroid hormone receptor (34, 35). However, recently, 3, 3'-T_2 has been found to stimulate mitochondrial respiration in various rat tissues (23) and rT_3 may play a role in regulating actin polymerization in brain cells (24). Thus, the possibility that these T_4 metabolites may play a physiologic role in developing animal cannot be excluded. In addition, we have demonstrated that sulfoconjugation facilitates fetal to maternal transfer of thyroid hormones and their metabolites, sulfated 3,3'-T_2 in particular (18, 36). It is possible that uterine sulfotransferase may be involved in such a transfer process.

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