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Characterization of Iodothyronine Sulfotransferase Activity in Rat Liver*

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ABSTRACT

Sulfation is an important pathway in the metabolism of thyroid hormone because it strongly facilitates the degradation of the hormone by the type I iodothyronine deiodinase. However, little is known about the properties and possible regulation of the sulfotransferase(s) involved in the sulfation of thyroid hormone. We have developed a convenient method for the analysis of iodothyronine sulfotransferase activity in tissue cytosolic fractions, using radioiodinated 3,3′-diiodothyronine (3,3′-T_2) as the preferred substrate, unlabeled 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as the sulfate donor, and Sephadex LH-20 minicolomns for separation of the products. We found that iodothyronine sulfotransferase activity in rat liver cytosol is 1) higher in male than in female rats; 2) optimal at pH 8.0; 3) characterized (at 50 μM PAPS and pH 7.2) by apparent Michaelis-Menton (K_m) values for 3,3′-T_2 of 1.77 and 4.19 μM, and V_{max} values of 1.94 and 1.45 nmol/min per mg protein in male and female rats, respectively; 4) characterized (at 1 μM 3,3′-T_2 and pH 7.2) by apparent K_m values for PAPS of 4.92 and 3.80 μM and V_{max} values of 0.72 and 0.31 nmol/min per mg protein, in males and females, respectively; 5) little affected by hyperthyroidism in both male and female rats, but significantly decreased by hypothyroidism in males but not in females; and 6) not affected by short-term (3 days) fasting in both male and female rats, but significantly decreased by long-term (3 weeks) food restriction to one-third of normal intake in males but not in females. It is suggested that the higher hepatic iodothyronine sulfotransferase activity in male *vs.* female rats, as well as the decreases induced in males by hypothyroidism and long-term food restiction, represents differences in the expression of the male-dominant isoenzyme rSULT1C1. (*Endocrinology* **138:** 5136–5143, 1997)

SULFATION is a detoxification reaction, the purpose of which is to increase the water solubility of lipophilic substrates and, thus, to increase their excretion in bile and/or urine. Sulfation is catalyzed by a family of homologous sulfotransferases located in the cytoplasmic fraction of different tissues, such as liver, kidney, intestine, and brain (1–3). These enzymes sulfate the hydroxyl group of a variety of endogenous and exogenous compounds, using 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as sulfate donor. On the basis of substrate specificity and amino acid sequence homology, three sulfotransferase subfamilies have been recognized, *i.e.* phenol sulfotransferases, estrogen sulfotransferases, and hydroxysteroid sulfotransferases (1–3).

Although sulfation also increases the water solubility of thyroid hormone, it does not merely serve to facilitate the biliary and/or urinary excretion of the hormone. Instead, we have shown that sulfation accelerates the degradation of different iodothyronines by the type I iodothyronine deiodinase (D1) (4, 5). This enzyme is important for the peripheral conversion of the prohormone T4 by outer ring deiodination (ORD) to the active hormone T_3 but is also capable of catalyzing the inner ring deiodination (IRD) of T_4 and T_3 to the inactive metabolites rT_3 and 3,3′-diiodothyronine (3,3′-T_2), respectively (6, 7). The preferred substrate for this enzyme is rT_3, which is very rapidly converted by ORD to 3,3′-T_2. Although sulfation does not affect the deiodination of rT_3, it has dramatic effects on the deiodination of other iodothyronines (4, 5). The IRD of T_4 by rat D1 is augmented ≈200-fold after sulfation of this substrate, whereas the ORD of T_4 sulfate (T_4S) is completely blocked (4, 5). Also the IRD of T_3 sulfate (T_3S) is much faster than that of nonsulfated T_3, and this has been observed with human, rat, and dog D1 (4, 5, 8). In contrast to the inhibited ORD of T_4S, ORD of 3,3′-T_2 sulfate (3,3′-T_2S) by rat, dog, and human D1 is extremely fast (4, 5, 8).

Iodothyronine sulfates are neither deiodinated by the type II deiodinase (D2), which catalyzes the ORD of T_4 and rT_3, nor by the type III deiodinase (D3), which catalyzes the IRD of T_3 and T_4 (Refs. 6, 7, and 9 and T. J. Visser and E. Kaptein, unpublished observations). The purpose of the facilitated degradation of T_4S and T_3S by D1 remains an enigma. It has been speculated that the role of sulfation is especially important when D1 activity is low, *i.e.* during fetal development and nonthyroidal illness (10). These conditions are associated with dramatic increases in the plasma concentration of the different iodothyronine sulfates (11–19). In these situations, sulfation is a reversible pathway of thyroid hormone inactivation, since free iodothyronines may be liberated from the conjugates by action of sulfatases expressed in different tissues or by intestinal bacteria (10, 20–22). Since T_3S does not bind to the nuclear T_3 receptor, the conjugate is devoid of thyromimetic activity unless it is desulfated (23).

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Little is known about the properties of the sulfotransferase isoenzymes catalyzing the sulfation of iodothyronines, let alone about the regulation of their expression. Sekura *et al.* (24) have studied the sulfation of different iodothyronines by partially purified rat hepatic arylsulfotransferase (AST) I and IV, showing preference for 3,3′-T_2 as the substrate. More recent studies of Gong *et al.* (25), Hurd *et al.* (26), and Santini *et al.* (27) have focused on the sulfation of T_3 in rat liver cytosol, showing markedly higher hepatic T_3 sulfotransferase activities in male than in female animals. This was correlated with the sex-dependent pattern of GH secretion in rats, *i.e.* pulsatile in males and more constant in females (25). T_3 sulfation has also been demonstrated in rat brain and kidney (26) as well as in human liver and intestine (28, 29). In the present study, hepatic iodothyronine sulfotransferase activity was characterized in male and female rats using 3,3′-T_2 as the preferred substrate, and occasionally with T_3 as the substrate. In addition, the possible effects of hypothyroidism, hyperthyroidism, short-term fasting, and long-term food restriction on hepatic iodothyronine sulfotransferase activities in both sexes were determined.

Materials and Methods

Materials

[3′-^{125}I]T_3 was obtained from Amersham (Amersham, Little Chalfont, UK); T_3, PAPS, methimazole, HEPES, and dithiothreitol were from Sigma (St. Louis, MO); 3,3′-T_2 and 3-iodothyronine (3-T1) were obtained from Henning Berlin GmbH (Berlin, Germany); and Sephadex LH-20 was purchased from Pharmacia (Woerden, The Netherlands). 3,[3′-^{125}I]T_2 was prepared by radioiodination of 3-T1 as previously described (8).

Animals

Male and female Wistar rats were obtained from Harlan Sprague-Dawley (Zeist, The Netherlands) or bred locally. They were housed in a controlled animal room with a 14-h light, 10-h dark photocycle and were provided *ad libitum* with food and drinking water. All experiments, which have also been described previously (30, 31), were approved by the Animal Welfare Committee (DEC) of Erasmus University.

Thyroid state. Rats were made hypothyroid by treatment for 2 weeks with drinking water containing 0.1% (wt/vol) methimazole. Hyperthroidism was induced by treating rats for 7 days with daily ip injections of 10 μg T_4 per 100 g body wt, while control rats received injections with vehicle (30).

Nutrition state. At the start of the experiments, rats were 10 weeks old (mean body wt: male rats, 216 g; female rats, 163 g). Daily food intake of control rats was 24 g in males and 15 g in females. Acute effects of starvation were studied in rats completely deprived of food for 3 days. The long-term effects of food restriction were studied in rats that were provided with only one-third of normal food intake during 3 weeks (FR33), *i.e.* 8 g for males and 5 g for females. Control animals continued to have free access to food, and all animals were supplied with drinking water *ad libitum* (31).

At the end of the treatments (24 h after the last T_4 dose), rats were anesthetized with ether and decapitated. Livers were isolated, immediately frozen in liquid nitrogen, and stored at −80 C until further processing. Liver tissue was homogenized in 0.25 M sucrose, 10 mM HEPES, and 1 mM dithiothreitol, and cytosol was prepared and stored in aliquots at −80 C as previously described (30, 31). Protein was measured with the Bio-Rad protein assay (Bio-Rad, Veenendaal, The Netherlands) using BSA as the standard.

Sulfotransferase assay

Iodothyronine sulfotransferase activities were usually assayed by incubation of 1 μM 3,3′-T_2 or T_3 and 100,000 cpm of the ^{125}I-labeled compound for 30 min at 37 C with the indicated amounts of liver cytosol in the presence or absence (blank) of 50 μM PAPS in 0.2 ml 0.1 M phosphate (pH 7.2) and 2 mM EDTA. Identical results were obtained using phosphate buffer without EDTA or buffer containing 2 mM Mg^{2+} or Ca^{2+}. The reactions were started by addition of cytosol diluted in ice-cold buffer and stopped by addition of 0.8 ml 0.1 M HCl. The mixtures were applied to Sephadex LH-20 minicolumns (bed volume, 1 ml), equilibrated in 0.1 M HCl. Iodide, sulfated iodothyronines, and nonsulfated iodothyronines were successively eluted with 2× 1 ml 0.1 M HCl, 6× 1 ml ethanol/water (20/80, vol/vol), and 3× 1 ml ethanol/0.1 M NaOH (50:50, vol/vol), respectively. Fractions were collected and counted for radioactivity. Sulfation in complete reaction mixtures was corrected for minor radioactivity detected in the corresponding fractions of the blanks. The use of special racks for parallel collection of fractions from 16 columns and a 16-channel γ-counter, with processing of the data by computer, allowed the analysis of a large number of samples in a single experiment.

Statistical analysis

Results are presented as means ±SD or as means of triplicate determinations in a representative experiment. Where appropriate, differences between groups were evaluated statistically by unpaired or paired Student's *t* test or by ANOVA followed by Duncan's multiple range test.

Results

Characterization of rat hepatic iodothyronine sulfotransferase activity

Figure 1 shows the chromatography of acidified reaction mixtures after incubation of radioactive 3,3′-T_2 with male rat liver cytosol in the absence or presence of PAPS. After incubation without PAPS, no radioactivity was eluted from the Sephadex minicolumns with acidic (0.1 M HCl) or neutral (20% ethanol in water) solvent but only with alkaline solvent (50% ethanol in 0.1 M NaOH), typical for the chromatography of nonsulfated 3,3′-T_2 (32). After incubation in the presence of PAPS, substantial radioactivity was eluted with the neutral solvent, peaking in the same fractions as synthetic 3,3′-

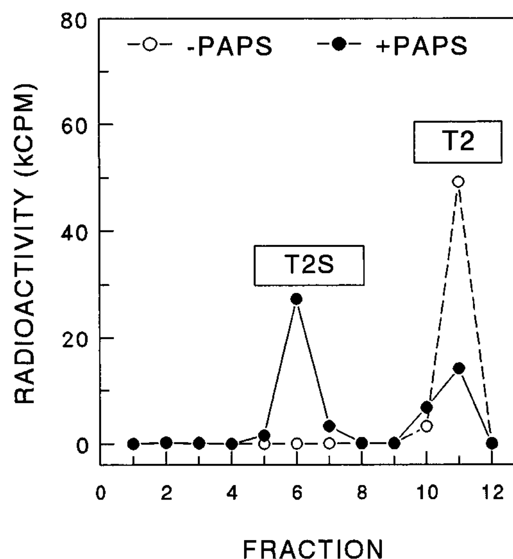

FIG. 1. Sephadex LH-20 analysis of acidified reaction mixtures after incubation of 1 μM 3,[3′-^{125}I]T_2 for 60 min at 37 C with male rat liver cytosol (25 μg protein/ml) in the absence or presence of 50 μM PAPS. After application of sample (1 ml), the minicolumns were successively eluted with 2× 1 ml 0.1 M HCl, 6× 1 ml ethanol-water (20:80, vol/vol), and 3× 1 ml ethanol-0.1 M NaOH (50:50, vol/vol).

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T_2S (32). More than 95% of applied 3,3′-T_2S was recovered in the neutral fractions. Similar separation was obtained between T_3 and T_3S (not shown). This method is generally applicable for separation of free and conjugated iodothyronines and is also used for fractionation of iodothyronine glucuronyltransferase assay mixtures (33). In the absence of added PAPS, no glucuronidation of 3,3′-T_2 was observed, which is not surprising since the uridine diphosphate-glucuronyltransferases are located in the microsomes, and no cofactor (uridine diphosphate-glucuronic acid) was added (33). Neither in the absence nor in the presence of PAPS was any radioiodide formation observed even though 3,3′-T_2S is a good substrate for D1, indicating that D1 activity is not present in rat liver cytosol but only in the microsomal fraction (6, 7).

Figure 2 compares the effects of increasing concentrations of unlabeled 3,3′-T_2 and T_3 on the sulfation of radioactive 3,3′-T_2 and T_3 by male rat liver cytosol in the presence of PAPS. Although the rate of T_3 sulfation was much lower than that of 3,3′-T_2, the dose-inhibition curves for unlabeled 3,3′-T_2 and T_3 were very similar if their effects on the sulfation of radioactive 3,3′-T_2 or T_3 were compared. In both cases, IC_{50} values were at least 10-fold lower for 3,3′-T_2 than for T_3. These results suggest that 3,3′-T_2 and T_3 are substrates for the same sulfotransferase isoenzyme(s), which is (are) more readily saturated by 3,3′-T_2 than by T_3.

Figure 3 shows the sulfation of 3,3′-T_2 by male and female rat liver cytosol in the presence of PAPS as a function of incubation time and cytosolic protein concentration. Under the same conditions, 3,3′-T_2 was sulfated more rapidly in male than in female rat liver. Regardless of gender, 3,3′-T_2 sulfation was linear with incubation time until ≈30% of the substrate was converted (Fig. 3A). With longer incubation times, sulfation rates leveled off probably due to substrate depletion. Since PAPS was added in large excess, depletion of the cofactor is unlikely. In both male and female rat liver, 3,3′-T_2 sulfation initially showed a more than proportional increase with the cytosolic protein concentration (Fig. 3B). For instance, an increase in the cytosolic protein concentration from 10 to 25 μg/ml resulted in a 4-fold increase in 3,3′-T_2S formation in both males and females. At higher protein concentrations, 3,3′-T_2 sulfation appeared to increase linearly with the protein concentration until significant substrate depletion occurred.

Figure 4 presents the effects of pH on the sulfation of 3,3′-T_2 by male and female rat liver cytosol in the presence of PAPS. At all pH values, the rate of 3,3′-T_2 sulfation was markedly higher in male than in female rat liver. In both sexes, highest 3,3′-T_2 sulfation rates were observed at pH 8. However, all subsequent experiments were carried out at the more physiological pH value of 7.2, providing sulfation rates that were ≈70% of those at the optimal pH.

Figure 5 shows the sulfation of 3,3′-T_2 by male and female rat liver cytosol at varying 3,3′-T_2 concentrations (0.5–10 μM) and a fixed PAPS concentration (50 μM). At all 3,3′-T_2 concentrations, sulfation rates were greater in male than in female rat liver. In both sexes, sulfation demonstrated saturation kinetics in the range of the 3,3′-T_2 concentrations tested. The double-reciprocal plots of sulfation rates *vs.* 3,3′-T_2 concentration were linear, allowing the calculation of apparent

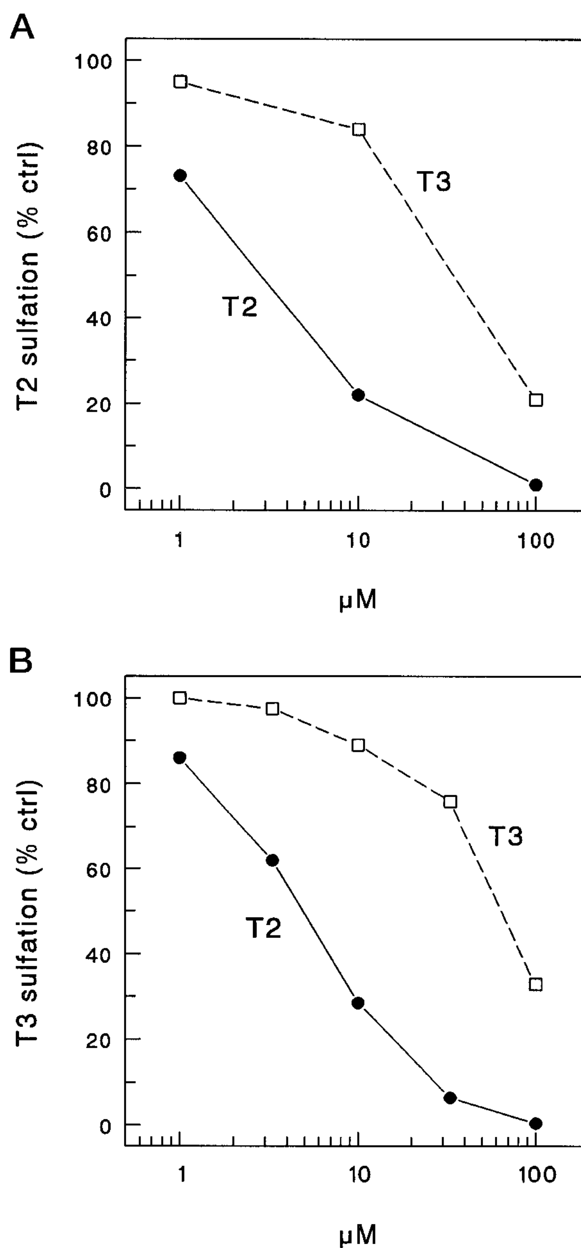

FIG. 2. A, Effects of increasing concentrations of unlabeled 3,3′-T_2 or T_3 on the sulfation of 3,[3′-^{125}I]T_2 by male rat liver cytosol. Reaction conditions: 25 μg cytosolic protein/ml, 50 μM PAPS, and 15 min incubation. B, Effects of increasing concentrations of T_3 or 3,3′-T_2 on the sulfation of [3′-^{125}I]T_3 by male rat liver cytosol. Reaction conditions: 0.25 mg cytosolic protein/ml, 50 μM PAPS, and 60 min incubation.

K_m values for 3,3′-T_2 and V_{max} values (at 50 μM PAPS). Table 1 presents the kinetic parameters derived from four experiments, showing that apparent K_m values for 3,3′-T_2 were significantly lower and V_{max} values somewhat higher in male than in female rat liver. From the data shown in Fig. 2, an apparent K_m value of 48 μM and V_{max} value of 0.22 nmol/min per mg protein were calculated for T_3 sulfation by male rat liver cytosol in the presence of 50 μM PAPS. Therefore, the apparent K_m value for T_3 is ≈30-fold higher and the V_{max} value ≈10-fold lower than the corresponding values for 3,3′-T_2 sulfation.

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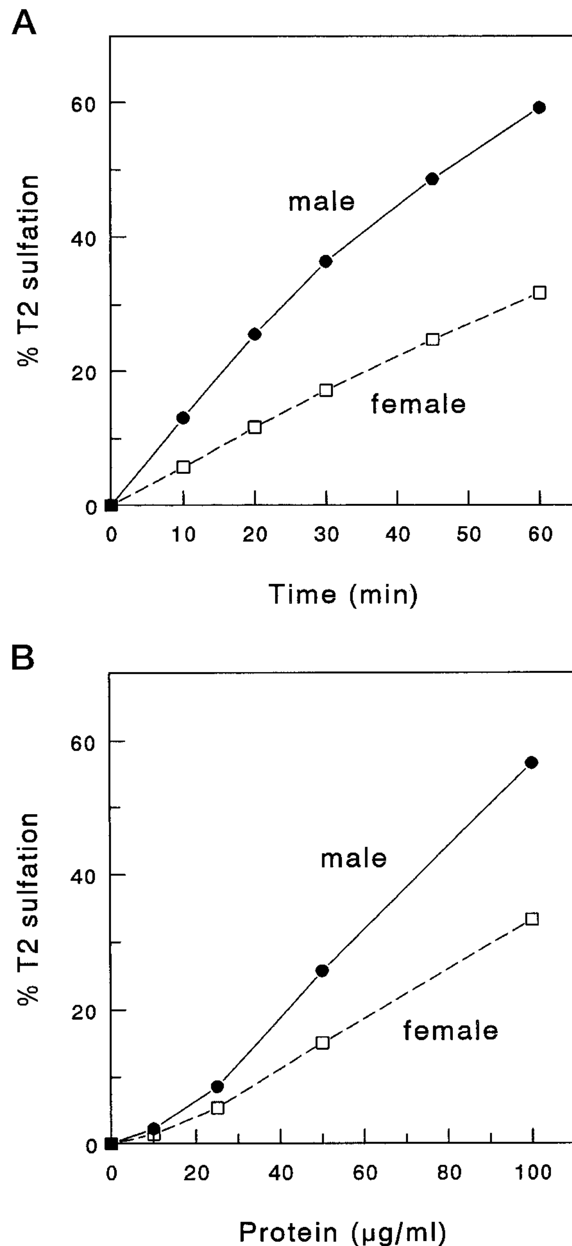

FIG. 3. A, Effects of incubation time on the sulfation of 1 μM 3,[3′-^{125}I]T_2 by male or female rat liver cytosol and 50 μM PAPS (25 μg protein/ml). B, Effects of protein concentration on the sulfation of 1 μM 3,[3′-^{125}I]T_2 by male or female rat liver cytosol and 50 μM PAPS (15 min incubation).

Figure 6 shows the effects of increasing PAPS concentrations (1–25 μM) on the sulfation of 1 μM 3,3′-T_2 by male and female rat liver cytosol. At all PAPS concentrations, 3,3′-T_2 sulfation rates were higher in male than in female rat liver cytosol. In both sexes, 3,3′-T_2 sulfation approached maximum rates at PAPS concentrations above 10 μM. The Lineweaver-Burk plots of these data were linear, from which apparent K_m values for PAPS and V_{max} values (at 1 μM 3,3′-T_2) were calculated. The kinetic parameters from three such experiments are presented in Table 1, showing that the apparent K_m value for PAPS is slightly higher while the V_{max} value is markedly higher in male than in female rat liver.

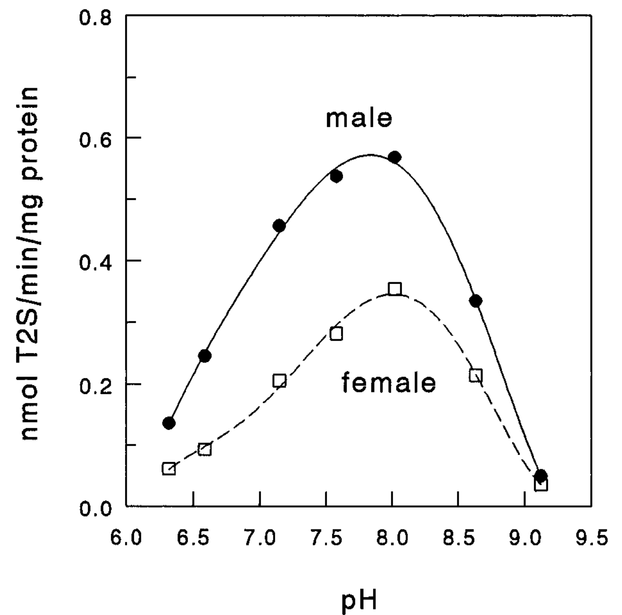

FIG. 4. Effects of pH on sulfation of 3,3′-T_2 by male or female rat liver cytosol. Reaction conditions: 1 μM 3,[3′-^{125}I]T_2, 25 μg cytosolic protein/ml, 50 μM PAPS, and 30 min incubation.

Regulation of rat hepatic iodothyronine sulfotransferase activity

Figure 7 presents the effects of thyroid state on hepatic 3,3′-T_2 sulfotransferase activities in male and female rats. The hypothyroid state of the methimazole-treated rats was demonstrated by marked decreases in serum T_4 and T_3 levels, as well as in hepatic D1 activities, and strong increases in serum TSH levels (30). Conversely, the hyperthyroid animals showed large increases in serum T_4 and T_3 levels, as well as in hepatic D1 activities, and marked decreases in serum TSH levels. In euthyroid controls, hepatic 3,3′-T_2 sulfotransferase activity was ≈2.5 times higher in males than in females. Methimazole-induced hypothyroidism was associated with a significant, 28% decrease in sulfotransferase activity in males but had no effect in females. Hyperthyroidism slightly decreased sulfotransferase activity by 12% in male rats but had no effect in female rats.

In both male and female rats, 3 days of fasting resulted in significantly decreased serum T_4, T_3, and TSH levels, as well as reduced hepatic D1 activities (31). Figure 8 shows that short-term fasting did not affect hepatic 3,3′-T_2 sulfotransferase activities in either males or females. A similar lack of effect of short-term fasting was observed if hepatic iodothyronine sulfotransferase activity was determined using T_3 as the substrate (not shown).

Like short-term fasting, long-term food restriction to one-third of normal intake (FR33) was associated with strong decreases in serum T_4, T_3, and TSH levels as well as in hepatic D1 activities in both male and female rats (31). Figure 9 shows the effects of FR33 on hepatic iodothyronine sulfotransferase activities determined with 3,3′-T_2 and T_3 as substrates. Food restriction resulted in a large, 51% decrease in 3,3′-T_2 sulfotransferase activity in male rats but had no effect in female rats, so that values were no longer different between food-

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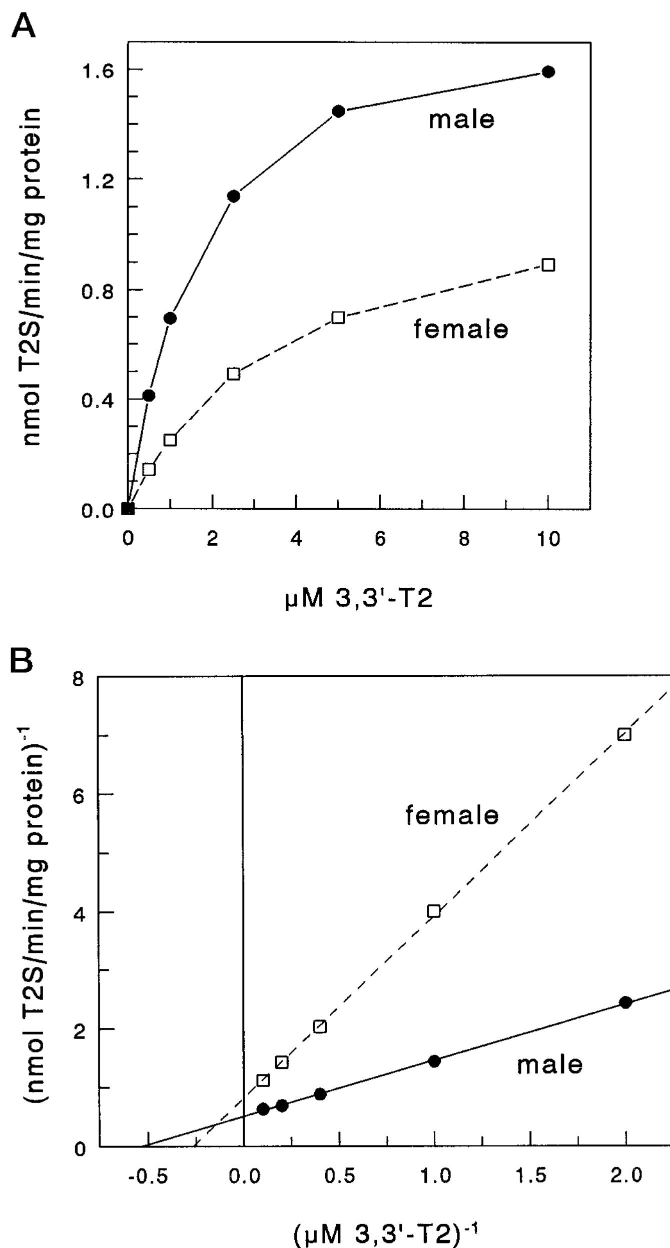

FIG. 5. Effects of substrate concentration on the sulfation of 3,3′-T_2 by male or female rat liver cytosol. A, linear plot; B, double-reciprocal plot. Reaction conditions: 0.5–10 μM 3,[3′-^{125}I]T_2, 25 μg protein/ml, 50 μM PAPS, and 15 min incubation.

TABLE 1. Kinetic parameters of 3,3′-T_2 sulfation by male and female rat liver cytosol[a]

Varying substrate	Parameter	n	Male	Female
3,3′-T2[b]	K_m[c]	4	1.77 ± 0.47	4.19 ± 0.56[d]
	V_{max}[e]	4	1.94 ± 0.38	1.45 ± 0.20
PAPS[f]	K_m[c]	3	4.92 ± 1.19	3.80 ± 0.76
	V_{max}[e]	3	0.72 ± 0.06	0.31 ± 0.04[d]

[a] Values are calculated from double-reciprocal plots, as illustrated in Figs. 5 and 6, and presented as means ± SD of the number of experiments indicated.
[b] Determined at 50 μM PAPS.
[c] Micromolar concentration.
[d] Significantly different from values in males: $p < 0.025$.
[e] Nanomoles/min per mg protein.
[f] Determined at 1 μM 3,3′-T_2.

restricted males and females (Fig. 9A). The sex-dependent difference in hepatic iodothyronine sulfotransferase activities in fed controls was even greater with T_3 (4.0-fold) than with 3,3′-T_2 (2.1-fold) as substrate. Long-term food resticition also resulted in a marked, 40% reduction in hepatic T_3 sulfotransferase activity in male rats but was without any effect in female rats. T_3 sulfation remained somewhat higher in food-restricted males than in females (Fig. 9B).

Discussion

For the determination of iodothyronine sulfotransferase activities in tissue cytosolic fractions, we have developed a method that uses radioiodinated 3,3′-T_2 as the preferred substrate, unlabeled PAPS as the sulfate donor, and Sephadex LH-20 minicolumns for the convenient isolation of the 3,3′-T_2S produced. Physiologically, T_3 is perhaps the most important substrate for iodothyronine sulfotransferase activity, since sulfation is an important pathway for the inactivation of the hormone. Sulfation not only nullifies the affinity of T_3 for its nuclear receptor (23), it also dramatically facilitates the degradation of the hormone by D1 (4, 5). Several lines of evidence indicate that 3,3′-T_2 is a preferred substrate for the same sulfotransferases that catalyze the sulfation of T_3. First, sulfation of 3,3′-T_2 by rat liver cytosol is inhibited by T_3, with an IC_{50} value similar to the apparent K_m value for T_3. Vice versa, sulfation of T_3 by rat liver cytosol is inhibited by 3,3′-T_2, with an IC_{50} value similar to the apparent K_m value for 3,3′-T_2. Second, sulfotransferase activities for T_3 and 3,3′-T_2 in rat liver show a similar sex dependence and are similarly affected by food deprivation (see below). Third, T_3 and 3,3′-T_2 have been directly shown to be sulfated by the same sulfotransferases purified from rat liver, *i.e.* AST I and AST IV (24), as well as by the same recombinant rat sulfotransferase isoenzymes, *i.e.* rSULT1B1 and rSULT1C1 (34–36). These findings indicate that 3,3′-T_2 and T_3 are indeed substrates for the same sulfotransferases, although sulfation of 3,3′-T_2 is catalyzed much more efficiently than sulfation of T_3. In male rat liver cytosol, sulfation of 3,3′-T_2 and T_3 are characterized by apparent K_m values of 1.8 and 48 μM, and V_{max} values of 1.9 and 0.22 nmol/min per mg protein, respectively. Therefore, the kinetic constant V_{max}/K_m, which determines the sulfation rate at low substrate concentration ($v/S = V_{max}/K_m$, if $S \ll K_m$) and, thus, is a measure of sulfation efficiency, is ≈200 times higher for 3,3′-T_2 than for T_3. Hurd *et al.* (26) reported a somewhat higher K_m value (114 μM) for T_3 sulfation by male rat liver cytosol and a much lower V_{max} value (0.16 nmol/h per mg protein). This may be explained, at least in part, by the use of a much lower PAPS concentration (0.4 μM) compared with our experiments (50 μM).

We found higher hepatic iodothyronine sulfotransferase activities with both 3,3′-T_2 and T_3 as substrate in male than in female rats. This is in agreement with previous findings reported by others (25–27). However, Gong *et al.* (25) showed that the sex dependence of hepatic sulfotransferase activity varies among species. Opposite to the situation in rats, he-

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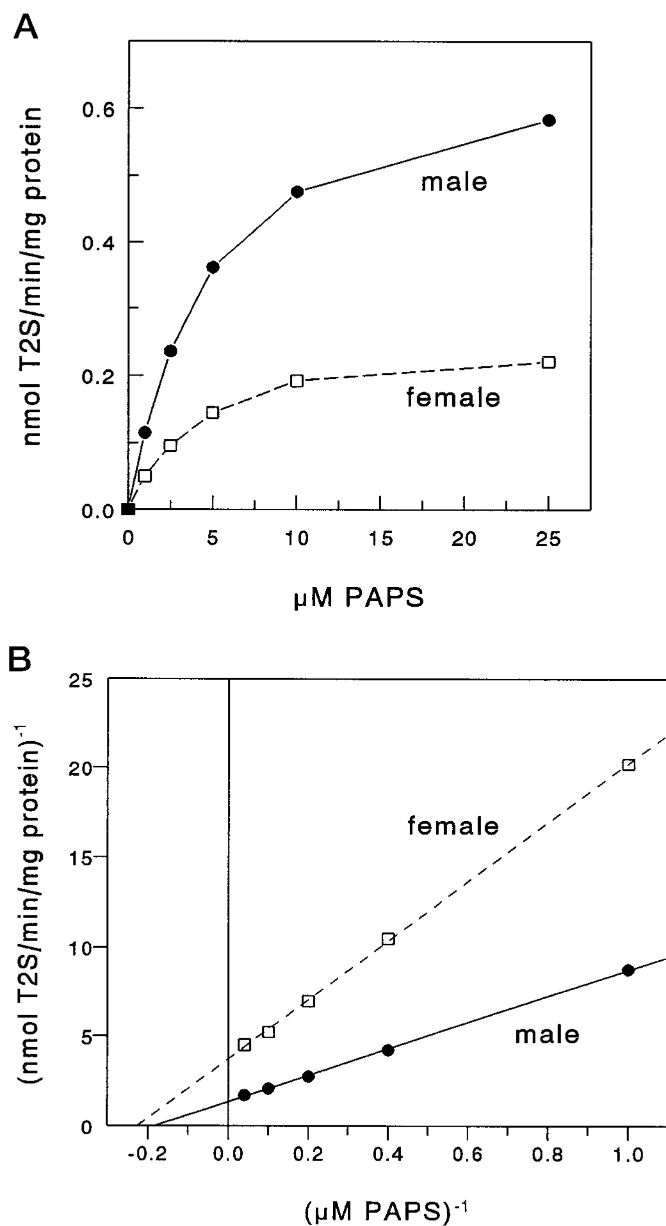

FIG. 6. Effects of cofactor concentration on the sulfation of 3,3′-T_2 by male or female rat liver cytosol. A, Linear plot; B, double-reciprocal plot. Reaction conditions: 1 μM 3,[3′-^{125}I]T_2, 25 μg protein/ml, 1–25 μM PAPS, and 15 min incubation.

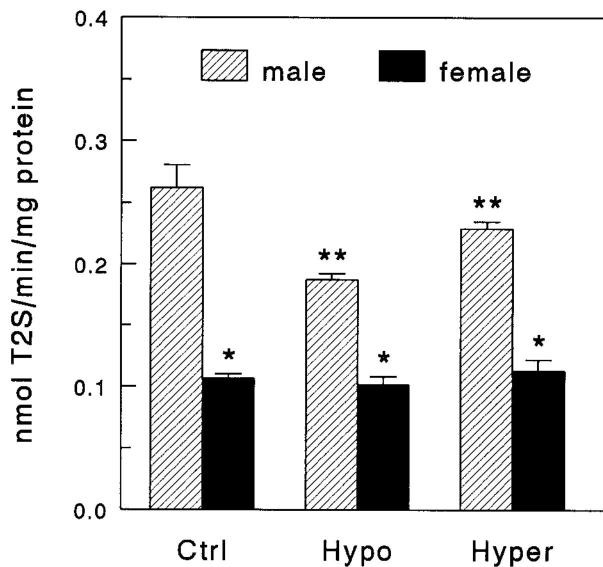

FIG. 7. Effects of methimazole-induced hypothyroidism and hyperthyroidism on hepatic 3,3′-T_2 sulfotransferase activities in male and female rats. Reaction conditions: 1 μM 3,[3′-^{125}I]T_2, 25 μg protein/ml, 10 μM PAPS, and 30 min incubation. Results represent the means ± SD of three to five rats per group. *, Significantly different from male rats, $P < 0.001$; **, significantly different from euthyroid controls, $P <$ 0.05 or less.

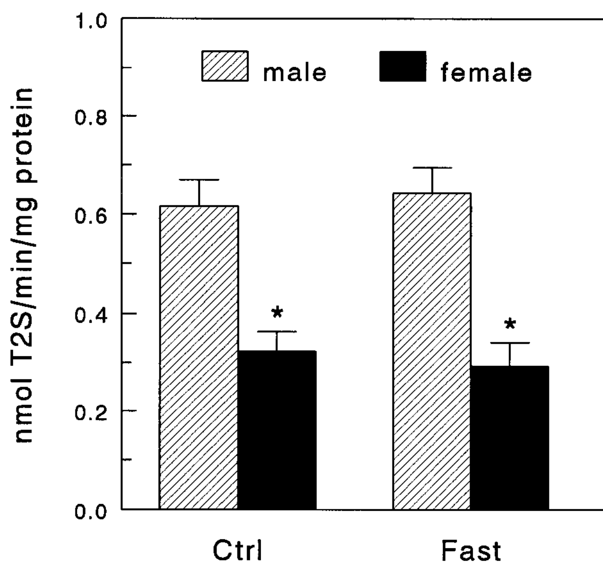

FIG. 8. Effects of short-term (3 days) fasting on hepatic 3,3′-T_2 sulfotransferase activities in male and female rats. Reaction conditions: 1 μM 3,[3′-^{125}I]T_2, 25 μg protein/ml, 10 μM PAPS, and 30 min incubation. Results represent the means ± SD of six rats per group. *, Significantly different from male rats, $P < 0.001$.

patic T_3 sulfotransferase activity is higher in female than in male mice, whereas no sex dependence is observed in humans. Gong *et al.* (25) have demonstrated that the higher T_3 sulfotransferase activity in male *vs.* female rat liver is not directly dependent on sex hormones. Instead, they found that this is determined by the different GH secretion patterns, being pulsatile in male rats and more constant in female rats. The group of Yamazoe (37) also provided evidence that the sex-dependent expression of certain cytochrome P450 isoenzymes in rat liver is also regulated by this difference in GH secretion pattern. The higher hepatic iodothyronine sulfotransferase activity in male *vs.* female rats is associated with higher serum levels of different iodothyronine sulfates in male than in female rats. This is not only true for the basal serum levels but in particular also for the increased serum levels of these conjugates observed in rats with impaired D1 activity due to selenium deficiency (38).

We found that the higher 3,3′-T_2 sulfotransferase activity in male than in female rat liver is associated with a small increase in V_{max} value as well as a larger decrease in apparent K_m value for 3,3′-T_2. These findings probably do not reflect true differences in K_m values, *e.g.* due to enzyme modifica-

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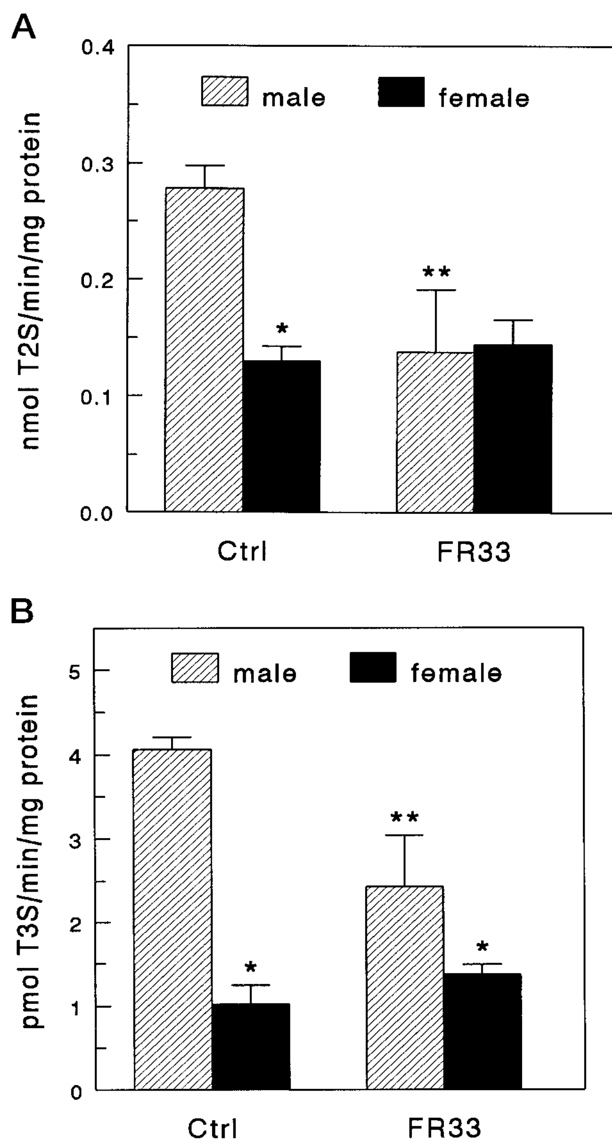

FIG. 9. Effects of long-term (3 weeks) food restriction (FR33) on hepatic sulfotransferase activities for 3,3′-T_2 (A) and T_3 (B) in male and female rats. Reaction conditions: A, 1 μM 3,[3′-^{125}I]T_2, 25 μg protein/ml, 10 μM PAPS, and 30 min incubation; B, 1 μM [^{125}I]T_3, 0.25 mg protein/ml, 50 μM PAPS, and 60 min incubation. Results represent the means ± SD of five rats per group. *, Significantly different from male rats, $P < 0.01$ or less; **, significantly different from fed controls, $P < 0.001$.

tion, but presumably represent differences in sulfotransferase isoenzyme composition. We and others have demonstrated that rSULT1B1 and rSULT1C1 are important isoenzymes for the sulfation of iodothyronines in rat liver, whereas rSULT1A1 does not catalyze iodothyronine sulfation (34–36). Expression of rSULT1C1 is much higher in male than in female rat liver, which has also been ascribed to the different GH secretion patterns in male and female rats (39, 40). In contrast, rSULT1B1 expression in rat liver appears to be independent of gender (34, 35). Therefore, sulfation of T_3 and 3,3′-T_2 in female rat liver probably represents predominantly the activity of rSULT1B1, whereas sulfation of these iodothyronines in male rat liver is catalyzed in addition by rSULT1C1. This suggests that the apparent K_m value for 3,3′-T_2 sulfation in female rat liver cytosol (4.2 μM) largely reflects the K_m value of rSULT1B1, whereas the apparent K_m value for 3,3′-T_2 in male rat liver cytosol (1.8 μM) represents a composite value, intermediate between the K_m values of rSULT1B1 and rSULT1C1. This is in agreement with our finding that the K_m value for 3,3′-T_2 sulfation by recombinant rSULT1C1 amounts to 0.75 μM (36); the K_m value for recombinant rSUL1B1 has not yet been determined. It should be mentioned that sulfotransferases may consist not only of two identical subunits but also of two different subunits (41). Dependence of sulfotransferase activity on homo- or heterodimer formation may explain our finding of a more than linear increase in 3,3′-T_2 sulfation rate with the cytosolic protein concentration.

Gong *et al.* (25) reported an increase in hepatic T_3 sulfotransferase activity in hyperthyroid male rats, whereas Hurd *et al.* (26) found no difference between normal and hyperthyroid animals. We did not observe an increase in hepatic 3,3′-T_2 sulfotransferase activity in hyperthyroid rats, although we found that hypothyroidism results in a significant decrease in males but not in females. In contrast to the lack of effect of short-term fasting on hepatic T_3 and 3,3′-T_2 sulfotransferase activities in both male and female rats, we observed a marked decrease in sulfotransferase activities for both substrates after long-term food restriction in males but not in females. Both hypothyroidism and food deprivation are known to be associated with a decreased GH secretion (42), where the effect of food deprivation may be mediated, at least in part, by the hypothyroid state of the (semi)starved animals. We therefore speculate that the male-specific decrease in hepatic iodothyronine sulfotransferase activity by both hypothyroidism and long-term food restriction is due to diminished expression of rSULT1C1 secondary to impaired GH secretion. Apparently, 3 days of fasting is not sufficient to produce a significant decrease in sulfotransferase expression.

In conclusion, we have developed a convenient method for the analysis of iodothyronine sulfotransferase activity in tissue cytoplasmic fractions. In agreement with previous reports, we found that this activity is higher in male than in female rat liver. We demonstrate that hepatic sulfation of thyroid hormone is not affected by hyperthyroidism and short-term fasting, whereas it is decreased by hypothyroidism and long-term food restriction in male but not in female rats. We speculate that the latter effects are mediated by an impaired GH secretion, resulting in diminished expression of the male-dominant isoenzyme rSULT1C1.

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