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Multiple UDP-glucuronyltransferases for the glucuronidation of thyroid hormone with preference for 3,3′,5′-triiodothyronine (reverse T_3)

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We have studied the glucuronidation of the thyroid hormones T_4, T_3 and rT_3 by liver microsomes of Wistar, Gunn and WAG rats. Gunn rats have a defect in the gene coding for bilirubin and phenol UDP-glucuronyltransferase (UGT) isoenzymes; WAG rats have a genetic defect in androsterone UGT. In normal Wistar rats UGT activity was ≈5-fold higher for rT_3 than for T_4 or T_3. UGT activities for T_4 and rT_3, but not for T_3, were impaired in Gunn rats. Conversely, UGT activity for T_3, but not for T_4 or rT_3, was impaired in WAG rats. Thus, in rat liver rT_3 is glucuronidated much more rapidly than T_4 and T_3. Our results support the view that T_4 and rT_3 are glucuronidated by bilirubin and phenol UGTs and T_3 by androsterone UGT.

Thyroid hormone; Iodothyronine; Glucuronidation; Bilirubin; Androsterone; Phenol; Rat strains

1. INTRODUCTION

Deiodination and conjugation are the principal metabolic pathways for thyroid hormone [1–3]. Thyroxine (3,3′,5,5′-tetraiodothyronine, T_4) is the main secretory product of the thyroid, but has little biological activity. The most active form of thyroid hormone, 3,3′,5-triiodothyronine (T_3), is largely produced by outer ring deiodination of T_4 in peripheral tissues. Conversely, if T_4 is deiodinated in the inner ring, the inactive metabolite 3,3′,5′-triiodothyronine (reverse T_3, rT_3) is produced. The type I iodothyronine deiodinase in liver and kidney plays an important role in the peripheral conversion of T_4 to T_3 but is much more active in the deiodination of rT_3 [1,2].

Hepatic T_4 glucuronidation is induced by treatment of rats with various substances, which has led to the hypothesis that T_4 is a substrate for at least two isoenzymes, i.e. 3-methylcholanthrene (MC)-inducible phenol UGT and clofibrate-inducible bilirubin UGT [4,5]. Glucuronidation of T_3 shows little response to these treatments. However, T_3 glucuronidation is impaired in Wistar LA, Fischer and WAG rats, all of which have a defect in androsterone UGT, while T_4 conjugation in these rats is virtually normal [4–6]. Gunn rats have a defect in the gene coding for multiple phenol and bilirubin UGT isoenzymes [7,8]. If our hypothesis about the glucuronidation of T_4 by these enzymes is correct, UGT activity for T_4, but not for T_3, should be impaired in Gunn rats. Also, to our knowledge rT_3 has never been tested as a substrate for microsomal UGT activity. We have, therefore, compared microsomal UGT activities for T_4, T_3 and rT_3 in untreated Wistar, Gunn and WAG rats.

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2. MATERIALS AND METHODS

2.1. *Tissue preparations*

Male rats weighing 150–200 g were used in all studies. Wistar and Gunn rats were obtained from Harlan Sprague–Dawley (Zeist, The Netherlands), and WAG/RIJ-MBL (WAG) rats were raised at TNO Medical Biological Laboratory (Rijswijk, The Netherlands). Animals were decapitated under ether anesthesia; the livers were rapidly isolated and stored at −80°C until further processing. For preparation of microsomes, tissues were homogenized on ice in 4 volumes 10 mM HEPES (pH 7.0), 0.25 M sucrose and 1 mM DTT. The homogenates were centrifuged for 10 min at 4°C and 25,000 × *g*, and the supernatants for 60 min at 100,000 × *g*. The microsomal pellets were dispersed in 0.1 M phosphate (pH 7.2), 2 mM EDTA and 1 mM DTT at a protein concentration of 10–20 mg/ml, and stored in aliquots at −80°C until further analysis. Protein content was determined with the BCA protein assay reagent (Pierce, Oud Beijerland, The Netherlands) using BSA as the standard.

2.2. *Iodothyronine UGT assay*

[3′,5′-^{125}I]T_4, [3′-^{125}I]T_3 and [3′,5′-^{125}I]rT_3 were obtained from Amersham (Amersham, UK). While [^{125}I]T_3 could be used without purification, labeled T_4 and rT_3 were purified before each assay on Sephadex LH-20 [4]. Nonradioactive T_4 and T_3 were obtained from Sigma (St. Louis, MO, USA) and rT_3 from Henning GmbH (Berlin, FRG). Iodothyronine UGT assay mixtures usually contained 1 μM T_4, T_3 or rT_3, ≈0.1 μCi of the ^{125}I-labeled substrate, 1 mg microsomal protein/

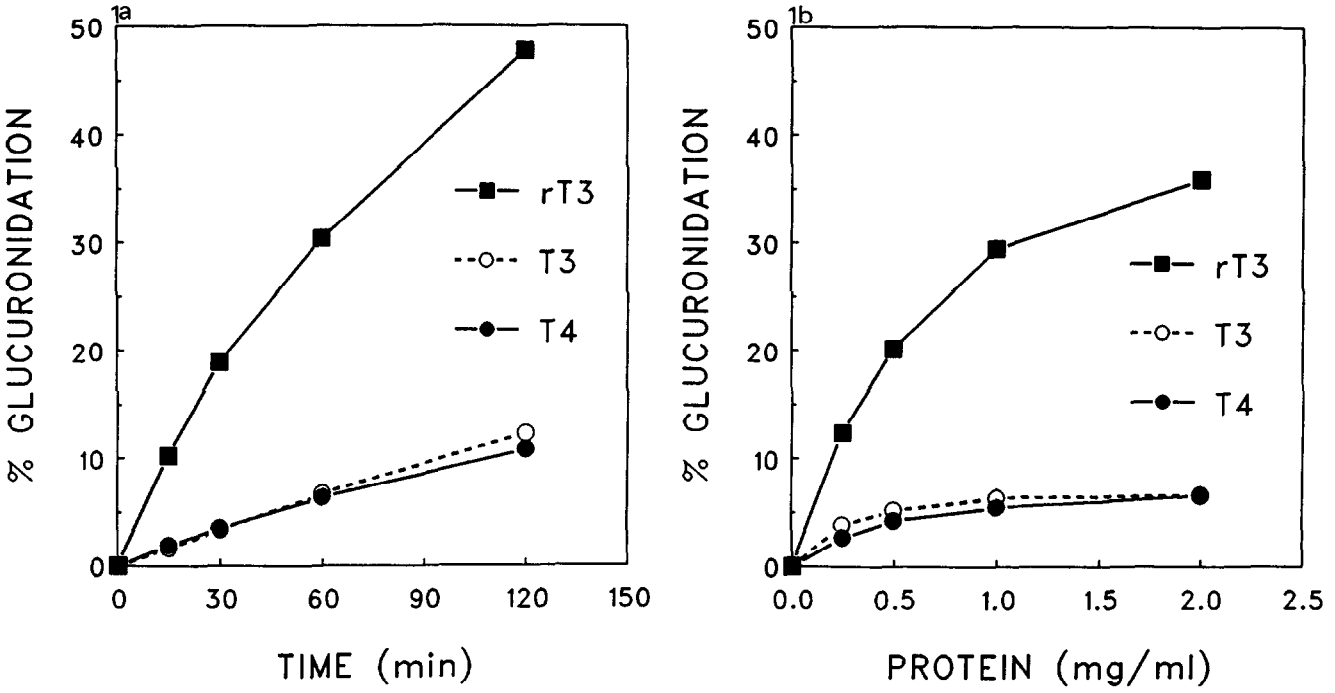

Fig. 1. Iodothyronine glucuronidation by Wistar HA rat liver microsomes. One μM T_4, T_3 or rT_3 were incubated in triplicate at 37°C during 15–120 min with 1 mg microsomal protein/ml (A) or during 60 min with 0.25–2 mg microsomal protein/ml (B).

ml, 1 mM propylthiouracil (PTU; Sigma), 5 mM UDP-glucuronic acid (UDPGA; Boehringer Mannheim, Almere, The Netherlands), 75 mM Tris-HCl (pH 7.8) and 7.5 mM $MgCl_2$, with a final volume of 200 μl. PTU was added to inhibit deiodination [1] without affecting the glucuronidation of the different iodothyronines. Incubations were done in triplicate for 1 h at 37C, and controls were incubated in the absence of UDPGA. Reactions were terminated by addition of an equal volume of ice-cold methanol. After centrifugation, supernatants were analyzed for glucuronide formation on Sephadex LH-20 [4].

2.3. *Other UGT assays*

PNP UGT activity was assayed by reaction of 1 mM PNP for 15 min at 37°C with 0.25 mg microsomal protein/ml and 5 mM UDPGA in 100 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$ and 0.005% Brij 58 [4,9]. Androsterone UGT activity was assayed by reaction of 100 μM androsterone for 15 min at 37°C with 0.5 mg microsomal protein/ml and 5 mM UDPGA in 100 mM Tris-HCl (pH 7.4), 3.75 mM $MgCl_2$ and 0.005% Brij 56. [4,10]. Bilirubin UGT activity was assayed using a modification of the method of Heirwegh et al. [11] by reaction of 100 μM bilirubin for 15 min at 37°C with 1 mg microsomal protein/ml and 5 mM UDPGA in 100 mM Tris-HCl (pH 7.8), 3.75 mM $MgCl_2$, 0.125% BSA and 0.025% CHAPS.

3. RESULTS

Determination of the androsterone UGT activity in the liver microsomes of the 6 Wistar rats tested showed that 4 animals had the high-activity (HA) and 2 the low-activity (LA) phenotype, glucuronidation rates in the latter being only 7% of those in the former. Table I presents the UGT activities for androsterone, PNP and bilirubin in the Wistar, WAG and Gunn rats. The mean androsterone UGT activity in the WAG rats is only 5% of that in Wistar HA rats and, thus, very similar to that in the Wistar LA rats. Androsterone glucuronidation rates were similar in Wistar HA and Gunn rats. Conversely, bilirubin glucuronidation was undetectable and PNP glucuronidation was strongly impaired in Gunn rats, while these UGT activities were similar in Wistar and WAG rats.

Fig. 1 shows the glucuronidation of T_4, T_3 and rT_3 by liver microsomes of Wistar HA rats as a function of the microsomal protein concentration and incubation time. The results indicate that the glucuronidation of T_4 and T_3 was roughly linear with time up to 2 h, while rT_3 glucuronide increased linear with time only during the first 30 min due to substrate depletion upon prolonged

Table I

Hepatic phenol, androsterone and bilirubin UGT activities in Wistar, Gunn and WAG rats.

Rats	UGT activity		
	PNP	Androsterone	Billirubin
Wistar	81.2 ± 9.6	6.13 ± 0.53[a]	0.77 ± 0.21
WAG	106.9 ± 18.6	0.29 ± 0.03[b]	NT[c]
Gunn	26.6 ± 6.6[b]	7.07 ± 0.26	0.00 ± 0.00[b]

Results are expressed as nmol/min/mg protein and presented as means ± S.D. of 4–8 determinations. [a]) Data represent values for 4 Wistar HA rats; androsterone UGT activity in 2 Wistar LA rats was 0.43 and 0.44 nmol/min/mg protein. [b]) Significant decrease compared with Wistar rats ($P < 0.001$). [c]) Not tested.

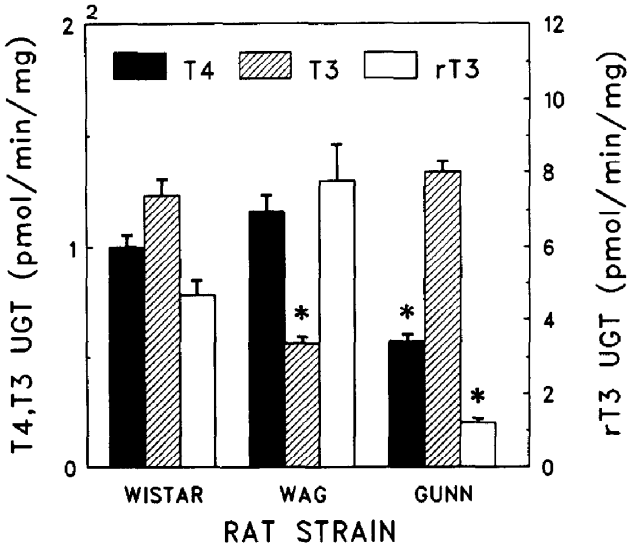

Fig. 2. Iodothyronine UGT activities in liver microsomes from Wistar HA, WAG and Gunn rats. One μM T_4, T_3 or rT_3 were incubated in triplicate for 60 min at 37°C with 1 mg microsomal protein/ml. Results are presented as mean ± S.D. of 4–6 determinations. *Significant decrease compared with Wistar HA rats ($P < 0.001$). Note the difference in scales for UGT activities for T_4 and T_3 (*left*) and for rT_3 (*right*).

incubation. Although the glucuronidations of T_4, T_3 and rT_3 were clearly dependent on the amount of microsomes added, the increases deviated from linearity at low protein concentration. It is clear, however, that at any incubation time or protein concentration glucuronide formation was similar with T_4 and T_3, while rT_3 glucuronidation was ≈ 5 times more rapid.

Fig. 2 shows the hepatic UGT activities for T_4, T_3 and rT_3 in Wistar HA, WAG and Gunn rats determined in standard 1 h incubations with 1 mg microsomal protein/ml. Compared with Wistar HA rats, glucuronidation of T_4 was on average decreased by 28%, of T_3 by 67%, and of rT_3 by 12% in the 2 Wistar LA rats (not shown). Mean UGT activities in WAG rats were 16% higher for T_4, 54% lower for T_3, and 66% higher for rT_3 than in Wistar HA rats. Mean glucuronidation rates in Gunn rats were 43% lower for T_4, 8% higher for T_3, and 74% lower for rT_3 in comparison with Wistar HA rats.

4. DISCUSSION

The glucuronidation of a variety of endogenous and exogenous compounds is catalyzed by a family of homologous UGT isoenzymes, which are located in the endoplasmic reticulum of liver as well as other tissues [12,13]. The purpose of this conjugation reaction is to increase the water-solubilty of the substrates and, thus, to expedite their excretion in the bile or the urine. In general, UGTs are high-K_m, high-capacity enzymes with broad and overlapping substrate specificities. The activities of some of these isoenzymes show specific responses to treatment of rats with microsomal enzyme inducers. For instance, clofibrate induces UGT activity for bilirubin but not for PNP, whereas MC induces UGT activity for PNP but not for bilirubin [12].

Recently, the structures of a number of UGTs have been characterized, among others those which glucuronidate phenols or bilirubin [13,14]. This has led to the recognition of the UGT1 subfamily in both rats and humans, which is comprised of multiple bilirubin and phenol UGT isoenzymes encoded by a single gene [8,13–15]. Through alternative splicing of the primary transcript, different mRNAs are produced, each of which combine a constant domain with a variable domain. These appear to code for the C-terminal UDPGA-binding domain and the N-terminal substrate-binding domain of the protein, respectively [8,13–15]. It has been demonstrated that Gunn rats have a −1 frameshift deletion in the common domain of the UGT1 gene, with a resultant failure in the expression of all members of this subfamily [7,8]. These rats, therefore, lack bilirubin and MC-inducible PNP UGT activities. The defective expression of androsterone UGT activity in Wistar LA rats has recently been found to be due to the deletion of a major portion of the gene [16,17]. This is probably also the case in WAG and Fischer rats, which are also deficient in androsterone UGT activity [5,6,18].

Previous observations have suggested that different UGT isoenzymes are involved with the glucuronidation of thyroid hormone. Thus, glucuronidation of T_4 in liver is stimulated by treatment of rats with different classes of microsomal enzyme inducers, such as (i) MC-type inducers, including PCBs, dioxin, β-naphtoflavone and hexachlorobenzene [4,6,19–28], (ii) phenobarbital [24–29], (iii) the phenoxyisobutyrate derivatives clofibrate, ciprofibrate and nafenopine [5,30,31], (iv) pregnenolone-16α-carbonitrile [25,27,28], and (v) spironolactone [32]. In general, the effects of such treatments on T_4 UGT activity are much greater than on T_3 glucuronidation, while studies of hepatic rT_3 UGT activity have not been reported .

Previous findings have indicated that rat strains with a genetic defect in androsterone UGT expression also show impaired T_3 UGT activity, while T_4 glucuronidation is not or only little affected [4–6]. A recent study has reported on a decrease in T_4 UGT activity in Gunn rats, but glucuronidation of T_3 and rT_3 was not analyzed [28]. We have now shown using liver microsomes of normal Wistar rats that glucuronidation rates are ≈ 5-fold higher with rT_3 than with T_4 or T_3 as substrate. In contrast to the marked impairment of T_3 UGT activity in WAG rats, glucuronidation of T_4 and rT_3 was not affected. Conversely, UGT activities for T_4 and especially rT_3 were markedly impaired in Gunn rats, whereas T_3 glucuronidation was normal.

Our results are compatible with the hypothesis that T_4 is conjugated by both MC-inducible phenol UGT

and fibrate-inducible bilirubin UGT [4,5]. Relative to T_4, however, rT_3 is by far the preferred substrate for these isoenzymes. These conclusions are supported by recent findings that T_4 and, even more so, rT_3 are glucuronidated by human phenol and bilirubin UGT clones, stably expressed in cell culture (manuscript in preparation). Androsterone UGT appears to be an important isoenzyme for the glucuronidation of T_3. However, the incomplete defects in the glucuronidation of T_4 and rT_3 in Gunn rats and that of T_3 in WAG rats suggest the involvement of additional UGT isoenzymes. It is remarkable that among the different iodothyronines rT_3 is most rapidly metabolized by both deiodination and glucuronidation. The importance of this for the regulation of thyroid hormone bioactivity remains to be fully understood.

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