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Glucuronidation of thyroid hormone by human bilirubin and phenol UDP-glucuronyltransferase isoenzymes

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The glucuronidation of thyroid hormone by UDP-glucuronyltransferases (UGTs) stably transfected in Chinese hamster V79 lung fibroblasts was investigated. Human bilirubin UGT (HP3) and phenol UGT (HP4) both catalysed the glucuronidation of T4 and rT3, whereas glucuronidation of T3 was not significant. rT3 was the preferred substrate for both isoenzymes, glucuronidation rates being 1.6- and 6.4-times higher than conjugation of T4 by HP3 and HP4 clones, respectively. This is the first identification of thyroid hormone as potential alternative endogenous substrate for bilirubin UGT.

Thyroid hormone: Iodothyronine; Glucuronidation; Bilirubin; Phenol; UDP-glucuronyltransferase; Human liver

1. INTRODUCTION

Deiodination and conjugation are the principal metabolic pathways for thyroid hormone [1–3]. Thyroxine (T4) is the main secretory product of the thyroid but has little biological activity. The most active form of thyroid hormone, 3,3',5-triiodothyronine (T3), is largely produced by outer ring deiodination of T4 in peripheral tissue, while the inactive metabolite, 3,3',5'-triiodothyronine (reverse T3, rT3), is generated by inner ring deiodination of T4 [1,2]. Glucuronidation is a major pathway for biotransformation of the iodothyronines [2,3], and the conjugates are subsequently excreted in bile.

Studies of rat strains with genetic defects in UGT isoenzymes have suggested that T4 and rT3 are primarily conjugated by both phenol and bilirubin UGTs and T3 by androsterone UGT [4–6]. Induction of hepatic microsomal phenol and bilirubin UGTs in rats by 3-methylcholanthrene (MC) and phenoxyisobutyrate (fibrate) compounds, respectively, led to an increase in the rate of glucuronidation of T4, also suggesting that this iodothyronine is a substrate for the two UGTs [5,7,8]. Further work has also correlated the induction of hepatic UGTs by xenobiotics with the reduction in serum thyroid hormone levels [9–11].

Little is known about glucuronidation of thyroid hormone in human liver or the possible changes which may be caused by chronic treatment with therapeutic drugs, which are inducers of UGTs. Therefore, in this study we have investigated the potential glucuronidation of T4, T3 and rT3 by human liver microsomes, as well as human phenol and bilirubin UGTs, produced by cloning cDNAs and stable expression in heterologous V79 cells, which do not exhibit endogenous background UGT activities [12–15].

2. MATERIALS AND METHODS

2.1. Materials

[3',5'-¹²⁵I]T4, [3'-¹²⁵I]T3 and [3',5'-¹²⁵I]rT3 were obtained from Amersham (Amersham, UK); T4 and T3 from Sigma (St. Louis, MO, USA), rT3 from Henning GmbH (Berlin, Germany); UDP-glucuronic acid (UDPGA) from Boehringer-Mannheim (Almere, The Netherlands); and Sephadex LH-20 from Pharmacia (Woerden, The Netherlands).

2.2. Transfected cells

Chinese hamster V79 lung fibroblasts were transfected with full-length HP1, HP3 or HP4 cDNA ligated into the eukaryotic expression vector pcDNA1neo as described in detail elsewhere [12–15]. As controls, non-transfected V79 cells were used. Transfected and control V79 cells were grown to 70% confluence, harvested, and washed twice with PBS. Cells were disrupted by hypotonic shock in water and repeated freeze–thawing, and the homogenates were stored 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. Human liver microsomes were obtained as previously described [16].

2.3. UGT assays

Glucuronidation of iodothyronines by human liver microsomes or cell homogenates was analyzed as previously described [4–7]. Briefly,

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1 μ M T4, T3, or rT3, and ≈ 0.1 μ Ci of the 125 I-labeled substrate were incubated in triplicate for 60 min at 37°C with 1 mg enzyme protein/ml and 5 mM UDPGA in 200 μ l 75 mM Tris-HCl (pH 7.8) and 7.5 mM MgCl₂. Control incubations were done in the absence of UDPGA. Reactions were terminated by addition of 200 μ l ice-cold methanol. After centrifugation, supernatants were analyzed for glucuronide formation on Sephadex LH-20 [4].

3. RESULTS

The present experiments have been conducted with three members of the UGT1 subfamily, i.e. HP1 which glucuronidates planar phenols such as 1-naphthol, HP3 which glucuronidates bilirubin, and HP4 which glucuronidates bulky phenols such as 2,6-diisopropylphenol [12–15]. The UGT activities of homogenates of V79 cells stably transfected with HP1, HP3 or HP4 cDNA, expressed per mg protein, are similar to the rates of glucuronidation of 1-naphthol, bilirubin and 2,6-diisopropylphenol, respectively, by human liver microsomes [12–15]. Another member of the UGT1 subfamily recently cloned and expressed in cell culture, HP2, also glucuronidates bilirubin [17], but has not yet been tested for iodothyronine UGT activity.

Human liver microsomes catalysed the glucuronidation of T4, T3 and rT3 at the rates shown in Fig. 1, representing 7% (T3, rT3) to 27% (T4) of the iodothyronine UGT activities determined previously in Wistar rat liver microsomes [6]. Glucuronidation of the different iodothyronines was undetectable in homogenates of non-transfected V79 cells, as well as in cells transfected with HP1 (not shown). Glucuronidation of T3 was also very low in homogenates of cells transfected with either HP3 or HP4 cDNA, however, these cells showed significant UGT activity for T4 and rT3 (Fig. 1). The HP3 and in particular the HP4 isoenzyme showed a clear substrate preference for rT3, glucuronidation rates of which were 1.6- and 6.4-fold higher than with T4, respectively.

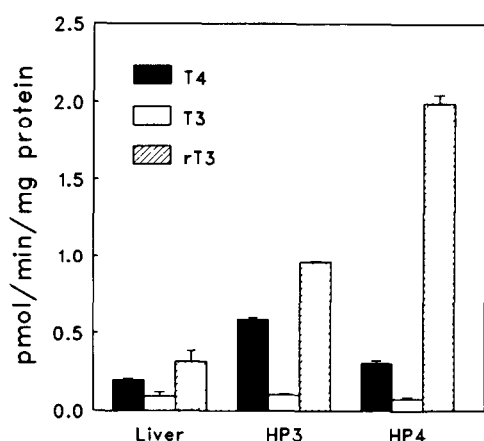


Fig. 1. UGT activities for T4, T3 and rT3 in human liver microsomes and in homogenates of V79 cells stably transfected with HP3 or HP4 cDNA. Results are the means \pm S.D. of 4 human livers or of triplicate incubations with cell homogenates in a representative experiment.

4. DISCUSSION

Glucuronidation is an important reaction for the metabolic clearance of a variety of endogenous and exogenous substances; it increases the water-solubility of these compounds and, thus, facilitates their biliary and urinary excretion [18,19]. The UGTs catalysing this reaction represent a family of homologous enzymes with broad and overlapping substrate specificity. They are located in the endoplasmic reticulum of different tissues, especially liver, and all use UDPGA as the glucuronic acid donor. Previous attempts to distinguish the different isoenzymes have been based on properties such as substrate specificity, ontogenic profiles and inducibility [18,19]. Recent progress in protein purification and cDNA cloning, however, have led to the elucidation of the structures of an increasing number of UGT isoenzymes [20].

Our findings fully support the hypothesis of the involvement of both phenol and bilirubin UGTs in the glucuronidation of T4 and rT3. Also in agreement with earlier work using rat liver microsomes [6] are the findings that rT3 is preferred over T4 as the substrate for these isoenzymes, in particular the phenol-glucuronidating UGT HP4. This is the first report of the identification of alternative endogenous substrates for bilirubin UGT. The insignificant glucuronidation of T3 by both phenol UGT and bilirubin UGT is also in agreement with previous findings [6]; however, the ultimate proof that T3 glucuronidation is indeed catalysed by androsterone UGT must await the availability of transfected cells, expressing high levels of this isoenzyme. Also, the possible contribution of the other bilirubin-conjugating isoenzymes HP2 to the glucuronidation of thyroid hormone remains to be investigated.

An exciting recent development has been the characterization in both humans and rats of the UGT1 subfamily, which comprises the 2 bilirubin UGTs (HP2 and HP3), the 2 phenol UGTs (HP1 and HP4) and at least 3 other isoenzymes, all encoded by a single gene [21]. Through differential splicing of the primary transcripts, the different mature mRNAs are produced, the sequences of which consist of a common domain and a variable domain. These probably code for the C-terminal UDPGA-binding domain and the N-terminal substrate-binding domain of the protein, respectively [19–21].

Although encoded by a single gene, the expression of the individual UGT1 isoenzymes in rats and possibly in man is subject to different control mechanisms. Thus, the expression of the phenol-conjugating isoenzyme(s) is increased by MC-type inducers, while the expression of the bilirubin UGT(s) is stimulated among others by fibrate derivatives [18]. UGT1 isoenzymes are also regulated by thyroid hormone in rats; bilirubin UGT activity decreases and phenol UGT increases in hyperthyroidism, while the reverse is true in hypothyroidism [22].

Since iodothyronines share UGTs with other endogenous substrates, such as bilirubin and perhaps androsterone, it may not be surprising that the role of changes in these UGT activities in the regulation by thyroid hormone of its own metabolism is limited [23].

In summary, we have demonstrated that T4 and rT3, but not T3, are substrates for both human UGT isoenzymes HP3 and HP4, which are known to glucuronidate bilirubin and bulky phenols, respectively. These results are in agreement with results in rats showing a parallel induction in UGT activities for bilirubin, T4 and rT3 after treatment with fibrates [5,7,8]. The increased glucuronidation of 1-naphthol, T4 and rT3 after treatment with MC-type inducers [4,6–8], probably reflects the simultaneous induction of the rat homologs of HP1 and HP4.

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