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MALE RAT HEPATIC UDP-GLUCURONOSYLTRANSFERASE ACTIVITY TOWARD THYROXINE

ACTIVATION AND INDUCTION PROPERTIES—RELATION WITH THYROXINE PLASMA DISAPPEARANCE RATE

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Abstract—Detergent-activation of UDP-glucuronosyltransferase (UGT) isoenzyme(s) involved in thyroxine (T4) glucuronidation in control, phenobarbital (PB)- and 3-methylcholanthrene (3-MC)-treated rats showed that between the four tested detergents, i.e. Triton X-100, Brij 58, Lubrol Px and 3-(3-cholamidopropyl)dimethylammonio-1-propanesulfonic acid (CHAPS), optimal activation of T4 UGT was displayed by the zwitterion CHAPS. "Native" versus optimal detergent-activated T4 UGT activity determination revealed that the latency of T4 UGT in microsomes from 3-MC-treated rats was decreased while the latency of T4 UGT in microsomes from PB-treated rats was increased compared to control, and suggest that the UGT isoenzyme(s) involved in the hepatic glucuronidation of T4 is (are) different in PB-treated rats than in 3-MC-treated rats. After a 7-day treatment with 20 mg/kg 3-MC, the activity of T4 UGT was increased 5-fold when determined in "native" and 4-fold when determined in optimal detergent-activated microsomes compared to controls. After a 7-day treatment with 75 mg/kg PB, T4 UGT was equivalent to the control when determined in "native", and increased 1.3-fold when determined in optimal detergent-activated microsomes. The results thus extend evidence that both 3-MC and PB induce the synthesis of UGT protein(s) involved in the glucuronidation of T4, 3-MC being a strong and PB a weak inducer. Hyperthyroid and hypothyroid status, achieved respectively by a 7-day treatment with 100 μg/kg T4 or a 7-day treatment with 10 mg/kg of one of the antithyroid drugs propylthiouracile or methymazole, did not modify T4 UGT activity, suggesting that the isoenzyme(s) conjugating T4 in microsomes from control rats is (are) unlikely to be either 4-nitrophenol or bilirubin UGT isoenzymes. After 14 days of treatment with 75 mg/kg PB, the hepatic glucuronidation rate of T4 was not different from the control when enzyme activity was expressed per mg microsomal protein but was significantly increased 1.4-fold when expressed per whole liver. A significant (1.5-fold) increase in the ^{125}I-T4 plasma elimination rate was also observed in PB-treated rats compared to controls. A strong (3.6-fold) increase in the T4 glucuronidation rate was observed in rats treated with 5 mg/kg 3-MC for 14 days while the ^{125}I-T4 plasma elimination rate was equivalent to the controls. These results demonstrate that there is no direct relation between T4 UGT activity (and subsequent biliary secretion of T4-glucuronides) and T4 plasma clearance and suggest an important contribution of the intestinal exchangeable thyroid hormone pool to the maintenance of blood thyroid hormone levels.

UDP-glucuronosyltransferase (UGT,† EC 2.4.1.17) belongs to a family of enzymes involved in the conjugation of UDP-glucuronic acid (UDPGA) to a variety of chemicals, drugs and endogenous compounds (for review see Ref. 1). The enzymes are exclusively associated with the membranes of the cell, mainly those of the endoplasmic reticulum. This situation confers to the protein a strong phospholipid dependence, since any perturbation of the lipid bilayer or change in the membrane lipid composition can simultaneously affect its activity [1, 2]. UGTs are latent enzymes, needing activation (in general by detergents) to express maximal activity [1, 3].

Each isoenzyme has been characterized by its selective induction by drugs or by its substrate specificity. Glucuronidation of planar monohydroxylated substrates (4-nitrophenol, 1-naphthol) is preferentially enhanced after treatment with 3-methylcholanthrene (3-MC), whereas the protein which catalyses glucuronidation of bulkier molecules (monoterpenoid alcohols, morphine and to a lesser extent bilirubin, phenolphalein and certain steroids) is induced by phenobarbital (PB) [1]. Other compounds have been reported to induce UGTs such as clofibrate (UGT toward bilirubin) [4] and pregnenolone 16α carbonitrile or spironolactone (UGT toward digitoxigenin monodigitoxoside) [5].

Conjugation of the phenolic hydroxyl group with

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† Abbreviations: UGT, UDP-glucuronosyltransferase; UDPGA, UDP-glucuronic acid; CHAPS, 3-(3-cholamidopropyl)-dimethylammonio-1-propanesulfonic acid; PTU, propylthiouracil; PB, phenobarbital; 3-MC, 3-methylcholanthrene; MMI, methymazole; T4, thyroxine; T3, 3,5,3′-triiodothyronine; TSH, thyroid-stimulating hormone; T4 UGT, thyroxine UDP-glucuronosyltransferase.

glucuronic acid is also an important metabolic pathway for thyroid hormones, thyroxine (T4) and 3,5,3'-triiodothyronine (T3) being normally excreted in rat bile mainly as the glucuronides [6, 7]. Little is known about the UGT isoenzyme involved in the glucuronidation of thyroid hormone. A variety of substances have been reported to increase T4 biliary clearance, including 3-MC [8], benzpyrene [9], 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin [10], polychlorobiphenyls [11, 12] and PB [13]. Similarly, based on observed increases in T4 plasma clearance, increases in hepatic T4 glucuronidation have also been suggested to occur in rats treated with spironolactone [14], clofibrate analogs [15, 16], leukotriene D4 receptor antagonist L-649,923 [17] and imidazole SC-37211 [18].

Conversely, thyroid hormones have been shown to regulate UGTs. Thus, an isoenzyme-specific influence of thyroid hormones on UGTs could be demonstrated in animal models of hyper- [19–21] and hypothyroidism [21], with 4-nitrophenol glucuronoconjugation following serum T4 variations and bilirubin glucuronidation varying opposite to serum T4 variations in the rat, phenolphthalein glucuronidation being not affected. Since the changes in enzyme activities were observed in both detergent-activated and "native" microsomal preparations [21] and were not associated with modifications in lipid organization of microsomal membranes [20], increase or decrease in protein rather than enzyme latency have been suggested to be involved in the process.

We recently developed a sensitive HPLC method, suitable for the determination of the T4 glucuronidation rate in "native" microsomes from control rats [22]. Our interest in the further characterization of this isoenzyme in the rat prompted us to conduct the present study on (a) the detergent-activation properties of T4 UGT in microsomes from control, PB- and 3-MC-treated rats, (b) its inducibility by PB and 3-MC, (c) its regulation by the thyroid status and (d) its relation with T4 plasma disappearance rate.

MATERIALS AND METHODS

Materials. UDPGA was from Boehringer, Triton X-100 from Prolabo, Lubrol Px, Brij 58, 3-(3-cholamidopropyl) - dimethylammonio - 1 - propanesulfonic acid (CHAPS), 3-MC and L-T4 from Sigma, propylthiouracil (PTU) from Fluka, PB from Rhône Poulenc and ^{125}I from NEN. The Amerlex kits used to determine T4, T3 and thyroid-stimulating hormone; (TSH) serum concentrations were from Amersham.

Animal treatment. Male Sprague–Dawley CD-BR rats (Charles River), 7 weeks old and weighing 200–250 g, were randomly allocated to large metal cages, with three rats per cage. Groups of rats, each consisting of six animals were treated p.o. (administration volume: 5 mL/kg) once daily as described previously [23]: 3-MC in corn oil at 20 mg/kg for 7 days and 5 mg/kg for 14 days; PB in saline at 100 mg/kg for 7 days and 75 mg/kg for 14 days; PTU in saline alkalinized with NaOH (pH 8.5) at 10 mg/kg for 7 days; methymazole (MMI) in methylcellulose at 10 mg/kg for 7 days and T4 in saline at 100 μg/kg for 7 days. Respective control animals were administered with the appropriate vehicle.

Serum T3, T4 and TSH concentrations. Serum hormone concentrations were measured by radioimmunoassay using the Amerlex kits as described previously [23].

Microsomal preparations. Control and treated rats were killed and exsanguinated. Hepatic microsomes were prepared by successive differential centrifugations as described previously [23], aliquoted and frozen at −80° until analysis.

T4 UGT activity determinations. Microsomes, "native" and detergent-treated, were prepared as described previously [24]: solutions containing 8 mg/mL microsomal proteins were preincubated at 37° for 10 min in the absence ("native" microsomes) and in the presence of Brij 58, Triton X-100, Lubrol Px at 0.2 mg/mg microsomal proteins or CHAPS at 0.6 mg/mg microsomal proteins, before being assayed for enzyme activity. T4 UGT activity was determined as described previously [22] with some modifications: the final assay mixture consisted of 0.5 mg/mL microsomal proteins, 50 mM phosphate buffer (pH 8.0), 10 mM magnesium chloride and 12.8 μM T4 containing 1 μCi/nmol ^{125}I-T4. The further steps were as described in Ref. 22.

^{125}I-T4 half-life determination. Determination of ^{125}I-T4 plasma elimination kinetics in rats treated with 75 mg/kg PB, 5 mg/kg 3-MC or 10 mg/kg were determined as described previously [23].

Statistical procedures. For statistical comparisons of data, the mean ± SD was calculated for each parameter and the treated groups were compared to the corresponding control group using either Student's *t*-test [25] when the variances were homogeneous according to Bartlett's test [26], or the non-parametric Wilcoxon signed rank test [27], when the variances were heterogeneous. The statistical significance of any difference from the corresponding control value was reported.

RESULTS

Detergent-activation of T4 UDP-glucuronosyltransferase in hepatic microsomes from control, PB and 3-MC-treated male rats

In a preliminary study, detergent-activation profiles of T4 UGT by three non-ionic detergents, Triton X-100, Brij 58 and Lubrol Px and by one zwitterion detergent, CHAPS have been determined [28], indicating that Brij 58, Triton X-100 and Lubrol Px displayed optimal activation of T4 UGT at a 0.2 detergent/protein ratio (mg/mg), while optimal activation of CHAPS occurred at a detergent/protein ratio of 0.6. Figure 1 shows the activation of T4 UGT in microsomes from control, 3-MC- and PB-treated rats. When microsomal preparations were incubated with Brij 58 or Triton X-100 at optimal activation concentrations, T4 UGT activity was increased to about 150% compared to "native" microsomes in both control and PB-treated rats. When incubated with Lubrol Px, T4 UGT activity was 180% compared to "native" in control and 220% compared to "native" in PB-treated microsomes. When activated by CHAPS, enzyme activity was

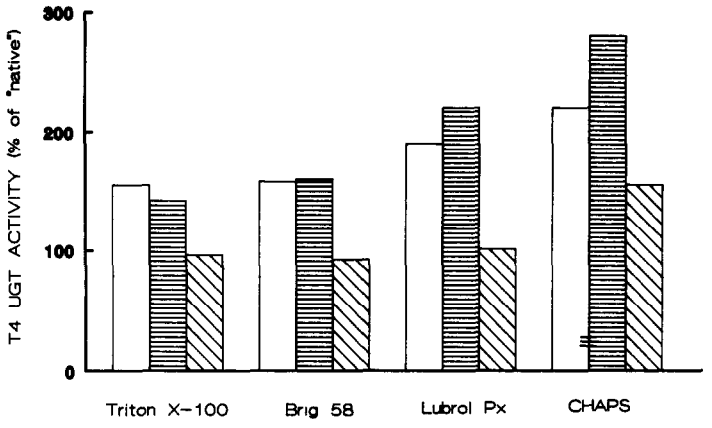

Fig. 1. T4 UGT activity was determined in "native" and optimal detergent-activated hepatic microsomes from control male rats (□) and male rats treated with 75 mg/kg PB (□) or 20 mg/kg 3-MC (▨) for 7 days. Detergent concentrations (mg/mg microsomal proteins) were 0.2 for Triton X-100, Brij 58 and Lubrol Px and 0.6 for CHAPS. T4 UGT activity was expressed as percentage of "native". Glucuronoconjugation rate (pmol/min/mg protein) in "native" microsomes was 7.1 ± 1.1 in control, 8.9 ± 0.05 in PB-treated rats and 42.8 ± 2 in 3-MC-treated rats. Values represent the mean ± SEM of three rats.

Table 1. Hepatic "native" and detergent-activated microsomal T4 UGT activity in male rats after 7 days of treatment with PB or 3-MC

	Hepatic T4 UGT activity (pmol/min/mg protein)	
Treatment	"Native" microsomes	Detergent-activated microsomes*
Control	7.62 ± 0.75	15.07 ± 1.53
PB (100 mg/kg)	8.07 ± 1.27	20.02 ± 2.46†
Control	11.07 ± 1.91	21.50 ± 3.02
3-MC (20 mg/kg)	50.55 ± 8.79‡	79.33 ± 5.93‡

Results are means ± SD of six rats/group.

* Detergent activation was performed using CHAPS (0.6 mg/mg microsomal proteins) as described in Materials and Methods.

† Significantly different from controls at the 1% level ($P < 0.01$).

‡ Significantly different from controls at the 1‰ level ($P < 0.001$).

220% and 280% of "native", respectively, in control and PB-treated microsomes. In microsomes from 3-MC-treated rats, T4 UGT could be activated only by CHAPS, with enzyme activity being 150% of "native" at the optimal detergent-activation concentration.

Effect of PB and 3-MC treatment on hepatic microsomal T4 UGT activity in male rat

A 7-day treatment with 100 mg/kg PB p.o. did not significantly increase T4 UGT activity compared to control when determined in "native" microsomes (Table 1). In contrast, a significant 1.3-fold increase of enzyme activity was evidenced in optimal detergent-activated (CHAPS 0.6 mg/mg protein) microsomes. A 7-day treatment with 20 mg/kg 3-MC p.o. induced a 4.6-fold increase of T4 UGT activity when determined in "native" microsomes and a 3.7-fold increase when determined in detergent-activated microsomes.

Effect of the thyroid status on hepatic microsomal T4 UGT activity in male rat

As expected, a 7-day treatment p.o. with 10 mg/kg of the antithyroid drugs PTU or MMI provoked a drastic decrease of both serum T3 and T4 (Table 2), inducing thus a hypothyroid status in the rats [21]. Conversely, and as expected, a 7-day treatment p.o. with 100 μg/kg T4 significantly increased both serum T3 and T4 concentrations, and thus induced a hyperthyroid status in the rats [19–21]. As shown in this table, thyroid status did not modify T4 UGT activity when determined either in "native" or optimal detergent-activated microsomes.

Table 2. Serum thyroid hormone concentrations and hepatic T4 UGT activity in male rats after 7 days of treatment with T4, PTU or MMI

Treatment	Serum thyroid hormones (nmol/L)		Hepatic T4 UGT activity (pmol/min/mg protein)	
	T4	T3	"Native" microsomes	Detergent-activated microsomes*
Control	33.85 ± 3.41	2.31 ± 0.48	6.92 ± 0.86	15.18 ± 1.75
PTU (10 mg/kg)	5.22 ± 1.90‡	<0.8‡	7.10 ± 0.56	15.50 ± 1.43
Control	36.65 ± 3.68	2.40 ± 0.49	6.22 ± 0.73	12.78 ± 0.72
MMI (10 mg/kg)	12.43 ± 4.33‡	0.80 ± 0.50†	6.27 ± 1.01	13.12 ± 0.60
Control	34.4 ± 5.6	1.93 ± 0.14	7.42 ± 1.03	14.52 ± 0.99
T4 (100 μg/kg)	77.5 ± 16.9†	5.66 ± 1.05†	6.93 ± 1.70	15.07 ± 1.53

Results are means ± SD of six rats/group.
* Detergent activation was performed using CHAPS (0.6 mg/mg microsomal proteins) as described in Materials and Methods.
† Significantly different from controls at the 1% level ($P < 0.01$).
‡ Significantly different from controls at the 1‰ level ($P < 0.001$).

Table 3. Serum T4, T3 and TSH after 14 days of treatment with PB or 3-MC

Treatment	Hormone concentration		
	T4 (pmol/L)	T3 (pmol/L)	TSH (ng/mL)
Control	39.19 ± 3.13	2.17 ± 0.21	6.82 ± 4.14
PB (75 mg/kg)	32.76 ± 4.16	1.73 ± 0.43	21.08 ± 8.87*
Control	37.88 ± 3.73	2.09 ± 0.50	7.19 ± 2.86
3-MC (5 mg/kg)	31.94 ± 3.83*	2.17 ± 0.48	13.45 ± 5.63*

Results are means ± SD of six rats/group.
* Significantly different from controls at the 5% level ($P < 0.05$).

Effect of PB and 3-MC treatment on serum T4, T3 and TSH concentrations

Table 3 shows that in rats treated for 14 days with 75 mg/kg PB, serum T3 and T4 hormone concentrations were slightly (15–20%) but not significantly decreased compared to control rats. In contrast, TSH was 3-fold increased. In rats treated for 14 days with 5 mg/kg 3-MC, serum T4 concentration was slightly (15%) and significantly decreased while T3 serum concentration was not significantly modified, TSH being about 2-fold increased.

Relation between T4 UGT activity and T4 plasma elimination rate in PB- and 3-MC-treated rats

The majority (60%) of T4 secreted into the bile is glucuronoconjugated [29]. Studies using varying substrates including T4 [9, 30] suggest that liver glucuronidation rate, and the subsequent biliary secretion of glucuronides *in vivo* is best approximated by T4 UGT activity when determined in "native" microsomes and when microsomal protein contents and liver weights are taken into account. Table 4 shows that in rats treated for 14 days with 75 mg/kg PB, the glucuronidation rate of T4 was not significantly different from control when enzyme activity was expressed per mg microsomal protein. However, the glucuronidation rate expressed per whole liver was 1.4-fold significantly increased in PB-treated rats compared to controls. As also shown in this table, a significant 1.5-fold increase of ^{125}I-T4 plasma elimination rate was observed in rats treated for 14 days with 75 mg/kg PB p.o.

A strong 3.6-fold increase of T4 glucuronidation rate was observed in rats treated with 5 mg/kg 3-MC p.o. for 14 days. However, ^{125}I-T4 plasma elimination rate was equivalent to controls.

DISCUSSION

As stated above, little is known about the isoenzyme involved in the glucuronidation of T4 in the rat. To get further insight into the characteristics of T4 UGT, we first studied the effect of PB and 3-MC treatment on T4 UGT activity and on its detergent-activation properties.

The activity of T4 UGT was strongly increased (about 4–5-fold) after 3-MC treatment, while it was poorly increased (1.3-fold) after PB treatment, and only when determined in detergent-activated microsomes (see Table 1). These results confirm those previously reported indicating that 3-MC [8] but not PB [9] increased T4 glucuronidation rate when determined using "native" enzyme. The 1.3-

fold increase of T4 UGT activity by PB treatment, observed in detergent-activated microsomes, confirm the results obtained by McClain *et al.* [13].

Between the four detergents tested, i.e. Triton X-100, Brij 58, Lubrol Px and CHAPS, the zwitterion CHAPS displayed maximal activation of T4 UGT, and this in microsomes from control as well as from PB- and 3-MC-treated rats. At optimal detergent-activation, T4 UGT activity in micosomes from PB-treated rats was higher compared to control microsomes, revealing an increase of UGT latency by PB treatment (see Fig. 1). This is further evidenced by the fact that T4 UGT activity, while not modified by PB treatment when determined in "native" microsomes, was significantly increased compared to control when determined in optimal detergent-activated microsomes (see Table 1). In contrast, optimal detergent-activated T4 UGT activity in microsomes from 3-MC-treated rats was equivalent to or lower compared to control microsomes. Added to the fact that the increase of T4 UGT activity after 3-MC treatment was higher when determined in "native" microsomes than when determined in detergent-activated microsomes, these results reveal that 3-MC decreased enzyme latency (see Fig. 1 and Table 1). It has already been reported that PB and 3-MC can modify UGT latency, the influence being isoenzyme-specific [24, 31, 32] and this can be accounted (a) to the reported alterations in lipid–protein interactions of the microsomal membrane by the two inducers [33, 34] and (b) to the well documented differences in the interaction with microsomal membranes of different isoenzymic forms of UGT [24, 25]. Our results thus suggest that the UGT isoenzyme(s) in microsomes from PB-treated rats involved in the glucuronidation of T4 are different from those in microsomes from MC-treated rats.

In addition, we [24] and others [20, 21, 26] have shown that enzyme activity determination in optimal detergent-activated microsomes best approximates the amount of protein present. Our results thus extend evidence that both 3-MC and PB induce the synthesis of UGT protein(s) involved in the glucuronidation of T4.

Glucuronidation toward T4 has rarely been evaluated directly and it is currently accepted that increases of UGT activity toward 4-nitrophenol is a good marker for the increase of T4 UGT activity [9, 13, 14, 17, 18]. We and others have indeed observed that 1-naphthol [24, 36] and 4-nitrophenol [31] UGT activity is strongly increased by 3-MC treatment but also significantly increased by PB treatment and this is due to the overlapping substrate specificity of some UGTs: at least three different UGT isoenzymes catalyse 4-nitrophenol (and 1-naphthol) glucuronidation, one of them being strongly induced by 3-MC, another one being the major isoenzyme for testosterone glucuronidation, thus being induced by PB [1]. However, increases in enzyme activity after PB treatment were observed in both "native" and detergent-activated microsomes when 1-naphthol and 4-nitrophenol were used as substrates [24, 31, 36] but only in detergent-activated microsomes when T4 was used as substrate (see Table 1). In addition, and in contrast to what has

Table 4. Hepatic microsomal T4 UGT activity and ^{125}I-T4 plasma elimination rate in male rats after 14 days of treatment with PB or 3-MC

Treatment	Hepatic T4 UGT activity			^{125}I-T4 plasma elimination	
	pmol/min/mg protein	pmol/min/g liver	nmol/min/whole liver	$T_{1/2}\beta$ (hr)	Cl × 10^4 (mL/min)
Control	7.93 ± 0.64	77.74 ± 12.83	1.04 ± 0.09	18.00 ± 0.59	28.88 ± 0.88
PB (75 mg/kg)	7.00 ± 1.56	83.01 ± 27.81	1.41 ± 0.29*	14.47 ± 1.27*	44.00 ± 2.04*
Control	2.56 ± 0.62	66.05 ± 6.80	0.92 ± 0.11	19.03 ± 5.33	29.49 ± 3.23
3-MC (5 mg/kg)	27.88 ± 7.86‡	226.50 ± 72.87†	3.32 ± 1.06†	16.84 ± 1.31	32.65 ± 3.87

Results are means ± SD of six rats/group.
$T_{1/2}\beta$, plasma elimination half-life.
Cl, plasma clearance.
* Significantly different from controls at the 5% level ($P < 0.05$).
† Significantly different from controls at the 1% level ($P < 0.01$).
‡ Significantly different from controls at the 1‰ level ($P < 0.001$).

been described for 1-naphthol/4-nitrophenol UGT [19–21], T4 UGT activity was not modulated by the thyroid status (see Table 2). These results make it unlikely that in control and PB-treated rats T4 and 4-nitrophenol glucuronidation are mediated by common isoenzyme(s).

Gunn rats are characterized by a complete absence of bilirubin glucuronoconjugation, a very reduced conjugation of 1-naphthol, 4-nitrophenol [1] and T4 [8], while testosterone and morphine conjugation is normal [1]. Because the glucuronidation of both T4 and bilirubin are impaired in Gunn rats and because T4 glucuronidation has been reported to be increased by fibrates [15, 16], it has recently been suggested that T4 could be a substrate for bilirubin UGT [12]. However, the insensitivity of T4 UGT to thyroid status (see Table 2) in contrast to bilirubin UGT [19–21], makes this unlikely.

In contrast to the finding in normal rats, UGT activity toward phenolic substrates [1] and T4 [8] is not increased in Gunn rats by 3-MC. Recently, it has been shown that a 3-MC-inducible UGT protein (called 4-nitrophenol UGT), contributing only minimally to the total 4-nitrophenol (and 1-naphthol) UGT activity in the liver of untreated normal rats [37], is absent in Gunn rats. As both 4-nitrophenol/1-naphthol [1, 24, 20, 36] and T4 (this study and Ref. 8) are strongly increased by 3-MC treatment in the rat, we suggest that the 3-MC-inducible 4-nitrophenol UGT is the isoenzyme mainly involved in T4 glucuronoconjugation in 3-MC-treated rats.

It is currently accepted that T4 plasma elimination rate is a good marker for biliary T4 clearance and thus for T4 UGT activity [13, 16–18, 38]. However, we previously noticed that a 14-day treatment of rats with 5 mg/kg 3-MC did not modify T4 plasma clearance [24], an observation also reported by Khanduja *et al.* [38]. In this study we show that the 1.4-fold increase in total liver T4 UGT activity observed in rats treated with 75 mg/kg PB for 14 days was accompanied by a 1.5-fold increase in serum T4 clearance. In contrast, although the 3-MC treatment strongly increased T4 UGT activity (3.5-fold, see Table 4), T4 serum disappearance rate was not significantly modified. The results obtained with 3-MC could be due to a lack of correlation between *in vitro* enzyme activity measurement and *in vivo* glucuronidation rate, through decreases by the treatment of hepatic UDPGA concentrations, the co-substrate for all glucuronidation reactions, playing a significant role in the *in vivo* regulation of glucuronidation. However this is unlikely, since it has been shown that hepatic UDPGA concentrations are in fact increased in rats after pretreatment with either PB or 3-MC [32]. The results more likely suggest that *in vivo* enzyme activity (and subsequent T4 biliary clearance) were increased in 3-MC (and PB)-treated rats, but that there was no direct relation between liver T4 biliary secretion and T4 plasma clearance. Indeed, it has recently been described that thyroid hormones T4 and T3 undergo enterohepatic circulation in the rat, with the conjugated hormones excreted in the bile being deconjugated in the gut and absorbed [29, 39]. These studies also established the intestine as an exchangeable hormone pool, controlling blood and tissue hormone levels. It seems therefore likely that the intestinal T4 absorption rate of 3-MC-treated rats in our study was such that the overall T4 plasma clearance was normal. Thus, both increased TSH secretion (see Table 3) and increased intestinal absorption could account for the only slight decreases in serum T4 levels observed in rats after 14 days of treatment with PB and 3-MC.

In summary, this study extends evidence that both 3-MC and PB induce the synthesis of UGT protein(s) involved in the glucuronidation of T4, 3-MC being a strong and PB only a weak inducer. Based on our results of latency, inducibility and response to the thyroid status, we suggest (a) that the isoenzyme(s) conjugating T4 in microsomes from control rats, unlikely to be either 4-nitrophenol or bilirubin UGTs, remain to be identified, (b) that these constitutive isoenzymes are weakly induced by PB and (c) that the 3-MC inducible 4-nitrophenol UGT is involved in the glucuronidation of T4 in microsomes from 3-MC-treated rats.

In addition, our results demonstrate that there is no direct relation between T4 UGT activity (and subsequent biliary secretion of T4-glucuronides) and T4 plasma clearance. They reveal that the contribution of the intestinal exchangeable thyroid hormone pool to the maintenance of blood thyroid hormone levels is important and has to be taken into account when the overall impact of T4 UGT induction on thyroid hormone homeostasis is discussed.

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