2. Ravindranath V, Anandatheerthavarada HK and
   Shankar SK, NADPH cytochrome P-450 reductase in
   rat, mouse and human brain. *Biochem Pharmacol* **39**:
   1013–1018, 1990.
3. Ravindranath V, Anandatheerthavarada HK and
   Shankar SK, Xenobiotic metabolism in human brain—
   presence and cytochrome P-450 and associated
   monooxygenases. *Brain Res* **496**: 331–335, 1989.
4. Anandatheerthavarada HK, Shankar SK and Rav-
   indranath V, Rat brain cytochromes P-450: catalytic,
   immunochemical properties and inducibility of multiple
   forms. *Brain Res* **536**: 339–343, 1990.
5. Ziegler DM, Microsomal flavin-containing mono-
   oxygenase: Oxygenation of nucleophilic nitrogen
   and sulpher compounds. In: *Enzymatic Basis of
   Detoxification* (Ed. Jakoby WB), Vol. I, pp. 201–207.
   Academic Press, New York, 1980.
6. Ziegler DM, Flavin-containing monooxygenases: cata-
   lytic mechanism and substrate specificities. *Drug Metab
   Rev* **19**: 1–32, 1988.
7. Ravindranath V and Anandatheerthavarada HK,
   Preparation of brain microsomes with cytochrome P-
   450 activity using calcium aggregation method. *Anal*

Biochem **187**: 310–313, 1990.
8. Cavagnaro J, Rauckman EJ and Rosen GM, Estimation
   of FAD-monooxygenase in microsomal preparations.
   Anal Biochem **118**: 204–211, 1981.
9. Bradford MM, A rapid and sensitive method for the
   quantitation of microgram quantities of protein utilizing
   the principle of dye binding. *Anal Biochem* **72**: 248–
   254, 1976.
10. Jeffcoate CRE, Gaylor JL and Calabrese RL, Ligand
    interaction with cytochrome P-450: binding of primary
    amines. *Biochemistry* **8**: 3455–3465, 1969.
11. Cashman JR and Hanzlik RP, Microsomal oxidation
    of thiobenzamide: a photometric assay for FMO.
    Biochem Biophys Res Commun **98**: 147–153, 1981.
12. Tynes RE and Hodgson E, Catalytic activity and
    substrate specificity of the flavin-containing mono-
    oxygenase in microsomal systems: characterization of
    the hepatic, pulmonary and renal enzymes of the
    mouse, rabbit and rat. *Arch Biochem Biophys* **240**: 77–
    93, 1985.
13. Duffel MW and Gillespie SG, Microsomal flavin-
    containing monooxygenase activity in rat corpus
    striatum. *J Neurochem* **42**: 1350–1353, 1984.

Differential expression and ciprofibrate induction of hepatic UDP-glucuronyltransferases for thyroxine and triiodothyronine in Fischer rats

The possible implication of thyroid hormone in the hypolipidaemic action of phenoxyisobutyrate (fibrate) drugs has been suggested by the finding that clofibrate stimulates liver mitochondrial α-glycerophosphate dehydrogenase activity, a classical thyromimetic response [1]. Observations of a drug-induced increase in hepatic uptake of thyroxine (T_4*) have been interpreted in support of this view [1]. Although clofibrate treatment raises the plasma level of T_4-binding proteins, competitive displacement of T_4 from these sites by the drug augments the plasma free T_4 fraction with a resultant shift into the tissues [1–3]. However, it is questionable if this results in a thyrotoxic state of the tissues, since the plasma free T_4 concentration during chronic clofibrate treatment is normal [2, 3].

Enzymatic deiodination determines the bioactivity of T_4 through conversion to the active hormone 3,3′,5-triiodothyronine (T_3) or to the inactive isomer 3,3′,5′-triiodothyronine (reverse T_3) [4]. Both metabolites are also further metabolized by deiodination. Other important pathways in the metabolism of thyroid hormone involve the conjugation of the phenolic hydroxyl group with glucuronic acid or sulfate [4]. The sulfate conjugates are

rapidly degraded in the liver by the type I iodothyronine deiodinase, and T_4 and T_3 are excreted in rat bile largely as glucuronides [4]. In contrast to an early report [5], clofibrate has been shown to stimulate the biliary clearance of T_4 [6]. Not only clofibrate [6, 7] but also fenofibrate [7], the fibrate derivative nafenopin [8] and ciprofibrate [9] accelerate the metabolic clearance of T_4 in rats. It has been demonstrated that nafenopin and ciprofibrate strongly increase the faecal clearance of T_4, whereas the urinary (deiodinative) clearance of T_4 is not affected [8] or even inhibited [9]. Little effect of these drugs was noted on the metabolic clearance of T_3 [8, 9]. Fibrates are well-known inducers of bilirubin UDP-glucuronyltransferase (UDPGT) in the liver [10, 11]. In this study we examined the effects of ciprofibrate administration to rats on hepatic T_4 and T_3 UDPGT activities. In order to gain more insight into the isozymes responsible for the glucuronidation of T_4 and T_3 we investigated, in parallel, the effects of ciprofibrate on p-nitrophenol (PNP) and androsterone UDPGT activities.

Materials and Methods

L-T_4, L-T_3, PNP and Brij 56 were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.), [^{125}I]T_4 (1500 μCi/μg) and [^{125}I]T_3 (2800 μCi/μg) from Amersham (Amersham, U.K.), androsterone from Steraloids (Wilton, NH, U.S.A.), [^{3}H]androsterone (116 μCi/μg) from New England Nuclear (Boston, MA, U.S.A.) and UDP-glucuronic acid (UDPGA) from Boehringer (Mannheim, F.R.G.). Fischer 344 rats were obtained from Charles River (Margate, U.K.) and Wistar rats from Harlan

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* Abbreviations: DTT, dithiothreitol; HA, high activity; LA, low activity; MC, 3-methylcholanthrene; PNP, p-nitrophenyl; PCB, polychlorobiphenyl; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; T_3, 3,3′,5-triiodothyronine; T_4, thyroxine; TBG, T_4-binding globulin; UDPGA, UDP-glucuronic acid; UDPGT, UDP-glucuronyltransferase.

Sprague Dawley (Zeist, The Netherlands).

Male Fischer 344 rats were treated orally for 14 days with ciprofibrate (30 mg/kg body wt per day) or with vehicle. Twenty-four hr after the last dose blood was taken for measurement of serum T_4 and T_3 concentrations. Livers were isolated, perfused with saline and homogenized in 0.25 M sucrose, 50 mM Tris–HCl (pH 7.4) and 1 mM DTT (25% w/w). Homogenates were centrifuged for 40 min at 9000 g and the supernatants for 60 min at 100,000 g. The resulting microsomal pellets were suspended in 0.25 M sucrose, 50 mM Tris–HCl (pH 7.4) and 1 mM DTT at a protein concentration of 10–20 mg/mL, and aliquots were stored at −70° until further analysis. Liver microsomes were prepared similarly from untreated male Wistar rats.

UDPGT activities were determined in duplicate at 37° in 200 μL 50–100 mM Tris–HCl (pH 7.4–7.8), 5–10 mM MgCl$_2$ and 0.05% Brij 56, with or without (blanks) 5 mM UDPGA as cofactor [10]. T_4 and T_3 UDPGT activities were assayed by incubation of 1 μM (≈0.1 μCi) [^{125}I]T_4 or [^{125}I]T_3 for 1 hr with 1 mg microsomal protein/mL. Reactions were stopped by addition of 200 μL ice-cold methanol and analysed by Sephadex LH-20 chromatography of the supernatants [12]. PNP UDPGT activity was assayed by incubation of 1 mM PNP for 30 min with 0.5 mg microsomal protein/mL. Reactions were stopped by addition of 3.8 mL 0.1 M NaOH and analysed by measuring the decrease in absorbance at 407 nm [13]. Androsterone UDPGT activity was assayed by incubation of 10 or 100 μM (≈0.1 μCi) [^{3}H]androsterone for 30 min with 0.25–0.5 mg microsomal protein/mL. Reactions were stopped by the addition of 2 mL ice-cold water and analysed by liquid scintillation counting after extraction of remaining substrate with ethyl acetate [14].

UDPGT activities in untreated rats of different strains were compared using analysis of variance followed by a test of least significant differences. The significance of differences in UDPGT activities between control and ciprofibrate-treated Fischer rats was determined using Student's t-test.

Results and Discussion

Treatment of Fischer rats with ciprofibrate produced a pronounced decrease in serum T_4 from 38.4 ± 4.5 (SD) to 7.7 ± 1.1 nmol/L (P < 0.001), while serum T_3 was not changed (0.55 ± 0.15 nmol/L in controls and 0.55 ± 0.08 nmol/L in ciprofibrate-treated rats). Analysis of the liver microsomes from these animals showed that ciprofibrate induced an approximately 3-fold increase in T_4 UDPGT activity and a 57% increase in T_3 UDPGT activity, whereas UDPGT activities for PNP and androsterone were decreased by 45% and 22%, respectively (Table 1).

We have recently observed a discontinuous variation in the T_3 UDPGT activity of Wistar rat liver microsomes which exactly matched the genetic heterogeneity of androsterone UDPGT in this rat strain [15]. The latter has been documented extensively by Matsui and Hakozaki [14], who showed that the difference between Wistar rats with low activity (LA) and those with high activity (HA) of androsterone UDPGT represents an autosomal recessive trait. It has recently been demonstrated that the defect is caused by a deletion mutation in the gene coding for this UDPGT [10]. T_4 glucuronidation is only slightly lower in LA compared with HA rats, and PNP UDPGT activity is not different between these phenotypes [15].

Figure 1 shows the comparison between the UDPGT activities for T_4, T_3 and androsterone in liver microsomes from untreated Fischer and Wistar rats. Hepatic PNP UDPGT activity in normal Fischer rats is about one-third of that in Wistar rats (25.6 ± 2.4 (SD) vs 74.1 ± 6.0 nmol/min/mg protein). Androsterone UDPGT activity in Wistar rat livers shows the well-established heterogeneity; under the assay conditions used, enzyme activity in LA microsomes is less than 10% of that in HA microsomes. Liver

Table 1. Effects of treatment of Fischer rats with ciprofibrate on liver microsomal UDPGT activities

Substrate	UDPGT activity*	
	Control	Ciprofibrate
T_4	1.61 ± 0.19	4.60 ±0.25‡
T_3	0.35 ± 0.05	0.55 ±0.03‡
PNP	25.6 ± 2.4	14.2 ±1.2‡
Androsterone†	86 ± 9	67 ±2‡

* Expressed as pmol (T_4, T_3, androsterone) or nmol (PNP) per min per mg protein (mean ± SD, N = 10).

† Assayed using 10 μM substrate and 0.5 mg microsomal protein/mL.

‡ Significantly different from controls (P < 0.001).

microsomal androsterone UDPGT activity in Fischer rats is the same as that in Wistar LA microsomes. T_4 is glucuronidated at roughly equal rates by liver microsomes from the different strains. However, T_3 glucuronidation shows a clear dichotomy with high activities in Wistar HA rats and low activities (≈30%) in Wistar LA and Fischer rats.

Several conclusions can be drawn from the present and previous findings concerning the identity of the UDPGT isozymes involved with the conjugation of thyroid hormone. (1) With an androsterone UDPGT activity as low as that in the Wistar LA phenotype, Fischer rats appear to have a constitutive defect in this isozyme. (2) Normally, T_3 is glucuronidated mainly by androsterone UDPGT or by another isoenzyme with an identically transmitted genetic defect. However, T_3 is also conjugated to some extent by an UDPGT isozyme which is induced by ciprofibrate [this study]. Glucuronidation of T_4 is not impaired in Wistar LA and Fischer rats, suggesting that it is largely conjugated by other isozymes than androsterone UDPGT. (3) Hepatic T_4 glucuronidation is stimulated by both 3-methylcholanthrene
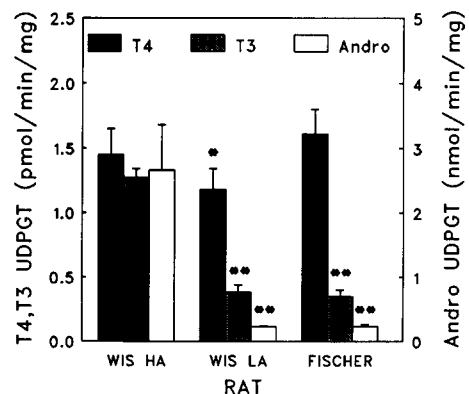

Fig. 1. UDPGT activities (mean ± SD) in liver microsomes from untreated Wistar HA (N = 8), Wistar LA (N = 4) and Fischer (N = 10) rats. Androsterone UDPGT activity was assayed using 100 μM substrate and 0.25 mg microsomal protein/mL. * Significantly different from Wistar HA rats, P < 0.05; **P < 0.001.

(MC)-type microsomal enzyme inducers such as MC itself [16], polychlorobiphenyls (PCB) [15, 17], and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) [18, 19], as well as by cipofibrate (this study). Studies with PCB and TCDD have shown that T_3 UDPGT activity is not increased [15, 18]. A selective induction of the metabolic clearance of T_4 and not of T_3 has recently also been found with the fibrate nafenopin [8]. Clofibrate and other fibrates appear to be specific inducers of bilirubin UDPGT [10, 11]. Therefore, these findings collectively suggest that T_4 is a substrate for both MC-inducible phenol UDPGT and clofibrate-inducible bilirubin UDPGT.

In conclusion, it has become clear that multiple UDPGT isozymes are involved with the glucuronidation of thyroid hormone. T_3 is perhaps primarily glucuronidated by androsterone UDPGT, whereas T_4 seems to be conjugated by both phenol and bilirubin UDPGT isozymes.

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REFERENCES

1. Ruegamer WR, Ryan NT, Richert DA and Westerfield WW, The effects of p-chlorophenoxyisobutyrate on the turnover rate and distribution of thyroid hormone in the rat. Biochem Pharmacol 18: 613–624, 1969.
2. McKerron CG, Scott RL, Asper SP and Levy RI, Effects of clofibrate (Atromid S) on the thyroxine-binding capacity of thyroxine-binding globulin and free thyroxine. J Clin Endocrinol 29: 957–961, 1969.
3. Lehotay DC, Paul HS, Adibi SA and Levey GS, Influence of clofibrate on thyroid hormone and muscle protein turnover. Metabolism 33: 1048–1051, 1984.
4. Visser TJ, Van Buuren JCJ, Rutgers M, Eelkman Rooda SJ and De Herder WW, The role of sulfation in thyroid hormone metabolism. Trends Endocrinol Metab 1: 211–218, 1990.
5. Osorio C, Walton KW, Browne CHW, West D and Whystock P, The effect of p-chlorophenoxyisobutyrate ('Atromid S') on the biliary excretion and distribution of thyroxine in the rat. Biochem Pharmacol 14: 1479–1481, 1965.

6. Harland WA and Orr JS, The effect of clofibrate on thyroxine metabolism. In: Thyroid Hormone Metabolism (Eds. Harland WA and Orr JS), pp. 65–87. Academic Press, London, 1975.
7. Hinton RH, Mitchell FE, Mann A, Chescoe D, Price SC, Nunn A, Grasso P and Bridges JW, Effects of phthalic esters on the liver and thyroid. Environ Health Perspect 70: 195–210, 1986.
8. Kaiser CA, Seydoux J, Giacobino JP, Girardier L and Burger AG, Increased plasma clearance rate of thyroxine despite decreased 5'-monodeiodination: study with a peroxisome proliferator in the rat. Endocrinology 122: 1087–1093, 1988.
9. Astley N, Deavy L, Harpur ES, Bonner FW, Lockwood G and Visser TJ, The effects of short term ciprofibrate administration on thyroid hormone metabolism in the male Fischer 344 rat. Human Exp Toxicol 10: 88–89, 1991.
10. Burchell B and Coughtrie MWH, UDP-glucuronyltransferases. Pharmacol Ther 43: 261–289, 1989.
11. Boiteux-Antoine AF, Magdalou J, Fournel-Gigleux S and Siest G, Comparative induction of drug-metabolizing enzymes by hypolipidaemic drugs. Gen Pharmacol 20: 407–412, 1989.
12. Rutgers M, Pigmans IGAJ, Bonthuis F, Docter R and Visser TJ, Effects of propylthiouracil on the biliary clearance of thyroxine (T_4) in rats. Endocrinology 125: 2175–2186, 1989.
13. Bock KW, Fröhling W, Remmer H and Rexer B, Effects of phenobarbital and 3-methylcholanthrene on substrate specificity of rat liver microsomal UDP-glucuronyltransferase. Biochim Biophys Acta 327: 46–56, 1973.
14. Matsui M and Hakozaki M, Discontinuous variation in hepatic uridine diphosphate glucuronyltransferase toward androsterone in Wistar rats. Biochem Pharmacol 28: 411–415, 1979.
15. Beetstra JB, Van Engelen JGM, Karels P, Van der Hoek HJ, De Jong M, Docter R, Krenning EP, Hennemann G, Brouwer A and Visser TJ, Thyroxine and triiodothyronine are glucuronidated in rat liver by different UDP-glucuronyltransferases. Endocrinology 128: 741–746, 1991.
16. Bastomsky CH and Papapetrou PD, The effect of methylcholanthrene on biliary thyroxine excretion in normal and Gunn rats. J Endocrinol 56: 267–273, 1973.
17. Bastomsky CH, Effects of a polychlorinated biphenyl mixture (Aroclor 1254) and DDT on biliary thyroxine excretion in rats. Endocrinology 95: 1150–1155, 1974.
18. Bastomsky CH, Enhanced thyroxine metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Endocrinology 101: 292–296, 1977.
19. Henry EC and Gasiewicz TA, Changes in thyroid hormones and thyroxine glucuronidation in hamsters compared with rats following treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 89: 165–174, 1987.

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