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**Short communications**

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

(Received 26 October 1990; accepted 9 March 1991)

The possible implication of thyroid hormone in the hypolipidaemic action of fenofibrate (fibrate) drugs has been suggested by the finding that clofibrate stimulates liver mitochondrial a-glycerophosphate dehydrogenase activity, a classical thyromimetic response [1]. Observations of a drug-induced increase in hepatic uptake of thyroxine (T4) have been interpreted in support of this view [1]. Although clofibrate treatment raises the plasma free T4 concentration [3], thyroxine; TBG, T4-binding globulin; UDPGA, UDP-glucuronic acid; UDPGT, UDP-glucuronoyltransferase.

Despite the finding that clofibrate stimulates liver mitochondrial a-glycerophosphate dehydrogenase activity, the possible implication of thyroid hormone in this action of clofibrate has not been explored in detail. However, it is questionable if this results in a thyrotoxic state of the tissues, since the plasma free T4 concentration remains normal [2, 3]. In this study we examined the effects of ciprofibrate administration to rats on hepatic T4 and T3 levels, and the metabolic properties of the hepatic UDP-glucuronoyltransferase for thyroxine and triiodothyronine.

Materials and Methods

We used male and female Fischer 344 rats, and Wistar strain rats from Harlan Laboratories, and male Sprague-Dawley rats from Charles River (Margate, U.K.) and U.S. Biochemical Co. (St Louis, MO, U.S.A.), [125I]T4 (1500 µCi/µg) and [125I]T3 (2800 µCi/µg) from Amersham (Amersham, U.K.), T3, T4, and 3,3',5-triiodothyronine, T3, T4, 3,3',5-triiodothyronine; T3, 3,3',5-triiodothyronine; T4, thyroxine; TBG, T4-binding globulin; UDPGA, UDP-glucuronic acid; UDPGT, UDP-glucuronoyltransferase.

* Abbreviations: DTT, dithiothreitol; HA, high activity; LA, low activity; MC, 3-methylcholanthrene; PNP, p-nitrophenol; PCB, polychlorobiphenyl; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; T3, 3,3',5-triiodothyronine; T4, thyroxine; Tg, T4-binding globulin; UDPGA, UDP-glucuronic acid; UDPGT, UDP-glucuronoyltransferase.
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 T₄, T₃ and androsterone 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 °C until further analysis. Liver microsomes were prepared similarly from untreated male Wistar rats.

UDPGT activities were determined in duplicate at 37 °C in 200 µL 50–100 mM Tris–HCl (pH 7.4–7.8), 5–10 mM MgCl₂, and 0.05% Brij 56, with or without (blanks) 5 mM UDPGA as cofactor [10]. T₄ and T₃ UDPGT activities were assayed by incubation of 1 µM (~0.1 µCi) [³²P]T₄ or [¹³¹I]T₃ for 1 h 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 using 1 mM PNP for 30 min with 0.5 mg microsomal protein/mL. Reactions were stopped by the 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) [³²P]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 T₄ from 38.4 ± 4.5 (SD) to 7.7 ± 1.1 nmol/L (P < 0.001), while serum T₃ 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 a 3-fold increase in T₄ UDPGT activity and a 57% increase in T₃ 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 T₄ 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]. T₄ glucuronidation is only slightly lower in LA compared with HA rats, and PNP UDPGT activity is not different between these phenotypes [15]. Androsterone UDPGT activity was assayed using 100 µM substrate and 0.25 mg microsomal protein/mL. * Significantly different from untreated rats (P < 0.001).

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 Wistar LA microsomes, T₄ is glucuronidated at roughly equal rates by liver microsomes from the different strains. However, T₃ glucuronidation shows a clear dichotomy with high activities in Wistar HA rats and low activities (~30%) in Wistar LA and Fischer rats.

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In conclusion, it has become clear that multiple UDPGT isozymes are involved with the glucuronidation of thyroid hormone. Therefore, these findings collectively suggest that T₃ is perhaps primarily glucurononidated by androsterone UDPGT, whereas T₄ seems to be conjugated by both phenol and bilirubin UDPGT isozymes.

REFERENCES


