ON THE EFFECT OF THYROID HORMONE ON 26-HYDROXYLATION OF C_{27}-STEROIDS IN RAT LIVER

I. BJÖRKHEM, H. DANIELSSON and J. GUSTAFSSON
Department of Chemistry, Karolinska Institutet, Stockholm, Sweden

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1. Introduction

The over-all rate of conversion of cholesterol into the main primary bile acids (cholic acid *plus* chenodeoxycholic acid) appears to be regulated by the activity of cholesterol 7α-hydroxylase [1]. The mechanisms by which the ratio of cholic acid to chenodeoxycholic acid is regulated have not been established. The only structural difference between cholic acid and chenodeoxycholic acid is the 12α-hydroxyl group in cholic acid and the activity of the 12α-hydroxylase could be of importance for the regulation of the ratio of cholic acid to chenodeoxycholic acid. In addition, a 26-hydroxylase might be of importance. Although 26-hydroxylation is an obligatory step in the biosynthesis of cholic acid as well as chenodeoxycholic acid it is known that at least in the rat introduction of 26-hydroxyl group prevents subsequent 12α-hydroxylation [1]. The possible roles of the 12α-hydroxylase and the 26-hydroxylase in the regulation of the ratio of cholic acid to chenodeoxycholic acid synthesized may be evaluated from experiments under conditions known to change the ratio between these acids in bile. Treatment with thyroid hormone leads to a marked decrease in the normal ratio between cholic acid and chenodeoxycholic acid in bile [2–5]. Treatment with propylthiouracil leads to an increase in this ratio, at least in rats with a biliary fistula [4]. The effect of thyroid hormone on 12α-hydroxylase has been previously studied [6]. Treatment with thyroid hormone led to a decrease in 12α-hydroxylase activity of about 40% whereas thyroidectomy increased the activity about 50%. These moderate effects on the 12α-hydroxylase do not appear to explain fully the marked changes in the ratio between cholic acid and chenodeoxycholic acid which are observed after treatment with thyroid hormone. The effect of thyroid hormone on the 26-hydroxylase has not been previously studied. The over-all side-chain oxidation of cholesterol by rat liver mitochondria [7] and the oxidation of 5β-cholestane-3α, 7α, 12α-triol by whole homogenate of rat liver [8] have been reported to be stimulated by thyroid hormone. As 26-hydroxylation is the first step in the degradation of the side chain, it is possible that the stimulatory effect of thyroid hormone is due to an effect on 26-hydroxylation. The 26-hydroxylase involved in bile acid biosynthesis has not been defined with certainty. It has been recently found that the mitochondrial and microsomal fractions of rat liver homogenate possess 26-hydroxylase activities with different properties and different substrate specificities [9, 10]. In the present report the effect of thyroid state on mitochondrial and microsomal 26-hydroxylase activity has been studied.

2. Methods

L-Triiodothyronine (50 μg/kg body weight in 0.1 ml of 0.9% sodium chloride solution) was administered to 200 g Sprague-Dawley rats by daily subcutaneous injections during four weeks. The control rats were given 0.1 ml of 0.9% sodium chloride solution. The oxygen consumption, expressed in ℓ per kg rat per hr [11], was 75% ($S_{\bar{x}}$ = 10%) higher in the triiodothyronine-treated groups than in the control groups. The control group gained in weight (final weight 300–350 g) whereas the triiodothyronine-treated group did not (final weight 200–250 g). Therefore, an additional control group was used in the tri-

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iodothyronine experiments that had the same weight as the treated rats (cf. table 1). Thyroidectomy of 200 g Sprague-Dawley rats was performed as described by Zarrow et al. [12]. Following surgery, the animals were given 1% calcium lactate to drink. The animals were killed one week after the surgery. The controls for the thyroidectomized rats were sham operated by making a midventral incision and exposing the sternohyoid muscle (cf. [12]). The thyroidectomy led to a decrease in oxygen consumption of 35% ($S_{\bar{x}}$ = 2%) as compared with the sham-operated group. Liver homogenates were prepared as described previously [10] using 0.25 M sucrose for the preparation of the mitochondrial fraction and 0.1 M Tris-Cl buffer, pH 7.4, for the preparation of the microsomal fraction. About half of the liver of each animal was used for the preparation of the mitochondrial fraction and half for preparation of the microsomal fraction. Incubation conditions and subsequent procedures for determination of mitochondrial and microsomal 26-hydroxylase activity were the same as described previously [10]. The mitochondrial 26-hydroxylase activity was assayed with [4-^{14}C]cholesterol as substrate and the microsomal 26-hydroxylase activity was assayed with 5β-[7β-^{3}H]cholestane-3α, 7α-diol as substrate. The analyses involve thin-layer chromatography followed by radio-gas chromatography of trimethylsilyl ethers of the products [10]. Microsomal 12α-hydroxylase activity (with 7α-hydroxy-4-[6β-^{3}H]cholesten-3-one as substrate) was assayed as described previously [6] with the exception that an incubation time of 20 min was used. In addition to analysis by thin-layer chromatography, the trimethylsilyl ethers of the products were quantitated by means of radio-gas chromatography using the same conditions as those for the determination of microsomal 26-hydroxylase activity [10].

3. Results and discussion

The results of the experiments with triiodothyronine-treated and thyroidectomized rats are summarized in table 1. The effect of triiodothyronine and thyroidectomy on microsomal 12α-hydroxylase activity was similar to that described previously [6]. Thus there was a small decrease in activity after treatment with triiodothyronine and a small increase after thyroidectomy. The mitochondrial 26-hydroxylase activity was not affected by thyroidectomy. Triiodothyronine treatment decreased the activity to about half when compared with control rats of the same weight. This effect might be due to the age of the animals as control rats of the same age as the triiodothyronine-treated rats had about the same mitochondrial 26-hydroxylase activity. The effects of triiodothyronine and thyroidectomy on microsomal 26-hydroxylase activity were opposite those on microsomal 12α-hydroxylase activity. Triiodothyronine treatment led to a more than two-fold increase of the activity whereas thyroidectomy decreased the activity to one fourth that of sham operated controls. It is noteworthy that the sham-operated animals used as controls for the thyroidectomized rats had a lower 12α-hydroxylase activity than the control animals used in the other experiments. This effect was apparently not due to hyperthyroidism as the sham-operated animals had an oyxgen uptake only about 10% higher than the other control animals. The ratio microsomal 12α-hydroxylase activity/microsomal 26-hydroxylase activity was about ten-fold lower in hyperthyroid rats than in hypothyroid rats. In contrast, the ratio microsomal 12α-hydroxylase activity/mitochondrial 26-hydroxylase activity was somewhat higher in hyperthyroid rats than in hypothyroid rats.

In the experiments shown in table 1,5β-cholestane-3α, 7α-diol was used as substrate for the microsomal 26-hydroxylase and 7α-hydroxy-4-cholesten-3-one as substrate for the microsomal 12α-hydroxylase. 7α-Hydroxy-4-cholesten-3-one is also 26-hydroxylated in the microsomes although to a considerably less extent than 5β-cholestane-3α,7α-diol [10]. However, 7α-hydroxy-4-cholesten-3-one could be a more important substrate for the microsomal 26-hydroxylase under conditions *in vivo* than 5β-cholestane-3α,7α-diol [10]. The ratio between microsomal 12α-hydroxylation and 26-hydroxylation of 7α-hydroxy-4-cholesten-3-one was found to be about 4 in control rats and about 1 in triiodothyronine-treated rats. In the experiments with thyroidectomized rats the rate of microsomal 26-hydroxylation of 7α-hydroxy-4-cholesten-3-one was too low to be detected with the gas-chromatographic technique used. Although the experiments were performed under conditions which might not have been optimal for microsomal 26-

Table 1
Effect of triiodothyronine and thyroidectomi on mitochondrial 26-hydroxylase activity and microsomal 12α- and 26-hydroxylase activity.

Preparation	Mitochondrial 26-hydroxylase activity	Microsomal 12α-hydroxylase activity	Microsomal 26-hydroxylase activity	Ratio microsomal 12α-hydroxylase activity/microsomal 26-hydroxylase activity
	(nmoles/mg protein)			
Controls of the same weight as triiodothyronine treated	0.8 ± 0.1	2.0 ± 0.2	1.9 ± 0.2	1.1
Controls of the same age as triiodothyronine treated	0.5 ± 0.1	1.6 ± 0.2	2.3 ± 0.4[a]	0.7
Triiodothyronine treated	0.4 ± 0.1	1.3 ± 0.2	4.7 ± 0.4[a]	0.3
Sham operated	0.9 ± 0.2	1.1 ± 0.1	2.5 ± 0.4	0.4
Thyroidectomized	0.7 ± 0.1	1.5 ± 0.2	0.6 ± 0.1	2.5

The values listed are the means of four experiments ± S.D. of the means with the exception of the values denoted with a) where eight experiments were performed.

hydroxylation of 7α-hydroxy-4-cholesten-3-one it appears that the effect of thyroid hormone on this reaction is the same as on microsomal 26-hydroxylation of 5β-cholestane-3α, 7α-diol.

The present results showing that the ratio microsomal 12α-hydroxylase activity/microsomal 26-hydroxylase activity differs by a factor of about 10 between the hyperthyroid state and the hypothyroid state should be compared with previous findings *in vivo*. The ratio cholic acid/chenodeoxycholic acid has been found to differ between the hyperthyroid state and the hypothyroid state by a factor of 3–7 in intact rats [3, 5] and 15–30 in rats with a biliary fistula [3, 4]. Since the effect of thyroid hormone on the ratio microsomal 12-hydroxylase activity/microsomal 26-hydroxylase activity is similar to the effect on the ratio cholic acid/chenodeoxycholic acid it may be concluded that the activity of both microsomal 12α-hydroxylase and microsomal 26-hydroxylase is important in the regulation of the ratio cholic acid/chenodeoxycholic acid in bile. The small effect of thyroid hormone on mitochondrial 26-hydroxylase activity suggests that this system is of minor importance in the biosynthesis of bile acids, at least in hyper- and hypothyroidism.

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References

[1] H. Danielsson, Mechanism of bile acid biosynthesis, in: The bile acids, eds. P.P. Nair and D. Kritchevsky (Plenum Press, 1973) in press.
[2] J.C. Thompson and H.M. Vars, Proc. Soc. Exp. Biol. Med. 83 (1953) 246.
[3] S. Eriksson, Proc. Soc. Exp. Biol. Med. 94 (1957) 582.
[4] O. Strand, Proc. Soc. Exp. Biol. Med. 109 (1962) 668.
[5] O. Strand, J. Lipid Res. 4 (1963) 305.
[6] K.A. Mitropoulos, M. Suzuki, N.B. Myant and H. Danielsson, FEBS Letters 1 (1968) 13.
[7] K.A. Mitropoulos and N.B. Myant, Biochem. J. 94 (1965) 594.
[8] O. Berséus, Acta Chem. Scand. 19 (1965) 2131.
[9] T. Cronholm and G. Johansson, European J. Biochem. 16 (1970) 373.
[10] I. Björkhem and J. Gustafsson, submitted to European J. Biochem. for publication (1973).
[11] E.G. Tomich and E.A. Woollett, J. Endocrinol. 11 (1954) 134.
[12] M.X. Zarrow, J.M. Yochim and J.L. McCarthy, in: Experimental Endocrinology (Academic Press, 1964) p. 240.