EFFECTS OF THYROIDECTOMY AND THYROXINE TREATMENT ON THE ACTIVITY OF 12α-HYDROXYLASE AND OF SOME COMPONENTS OF MICROSOMAL ELECTRON TRANSFER CHAINS IN RAT LIVER

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1. Introduction

Thyroid hormone increases the rate of formation of total bile acids in rats [1]. This effect is due mainly to increased production of chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanoic acid), with a slight inhibitory effect [2,3], or no effect [1], on the production of cholic acid (3α,7α,12α-trihydroxy-5-β-cholanoic acid). In this communication we show that the microsomal 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one, an intermediate in the biosynthesis of cholic acid from cholesterol [4,5,6], is decreased by thyroxine treatment and is increased by thyroidectomy. In an attempt to explain the effect of thyroid hormone on the 12α-hydroxylation required for cholic acid formation we have measured NADPH-cytochrome c reductase, cytochrome P-450 and cytochrome b_5 in the liver microsomes of our experimental animals, since all three factors have been shown to participate in the transfer of electrons from reduced pyridine nucleotides in microsomes.

2. Methods

Thyroxine (100 μg in 0.2 ml of 50% propylene glycol in water) was given daily by intraperitoneal injection and the rats were killed one day after the last injection; control animals were injected with 0.2 ml of propylene glycol solution at the same time. The thyroids of the hypothyroid rats were removed 17-21 days before the animals were killed; control animals were sham-operated at the same time. For each experiment, the livers were pooled from two or three treated animals and from an equal number of control animals, and were homogenized with 4 vol. of ice-cold 0.25 M sucrose. 12α-Hydroxylase activity was estimated from the amount of [^{3}H]-7α-hydroxycholest-4-en-3-one converted into 7α,12α-dihydroxycholest-4-en-3-one during a 10-min incubation in a microsomal suspension prepared from the liver homogenate by the method of Suzuki et al. [7]. The composition of the incubation mixture and the extraction and chromatographic analysis of the products of incubation were essentially those described by Danielsson and Einarsson [5]. NADPH-cytochrome c reductase activity was assayed by the method of Phillips and Langdon [8]. Cytochromes P-450 and b_5 were assayed by the method of Omura and Sato [9]. [^{3}H]-7α-hydroxycholest-4-en-3-one was prepared as described elsewhere [10].

3. Results and discussion

12α-Hydroxylating activity, NADPH-cytochrome c reductase and the concentrations of the two cytochrome pigments were assayed in four preparations from thyroidectomized rats, in five preparations from thyroxine-treated rats and in the controls appropriate for each group. Although the values from one

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Table 1

Effect of thyroidectomy on 12α-hydroxylase and NADPH-cytochrome c reductase activities, and on the concentrations of cytochromes P-450 and b_5 in rat-liver microsomes.

Preparation	12α-Hydroxylase	NADPH-c reductase	P-450	b_5
Control	0.383	182	0.975	0.307
Thyroidectomized	0.587	80	1.195	0.375

12α-Hydroxylase activity is expressed in mμmoles of 7α,12α-dihydroxycholest-4-en-3-one formed/mg protein/10 min incubation; NADPH-cytochrome c reductase activity is expressed in mμmoles of cytochrome c reduced/mg protein/min; the concentrations of P-450 and b_5 are expressed in mμmoles/mg protein.

experiment to another were rather variable, the effects of thyroidectomy and thyroxine treatment were very reproducible.

In all the preparations from thyroidectomized rats, 12α-hydroxylating activity was stimulated. The yield of 12α-hydroxylated product formed in the incubation mixture was 16.7 ± 1.2% of the amount of substrate added in the control preparations and 22.8 ± 1.9% in the preparations from the thyroidectomized animals. In agreement with the findings of other workers, the activity of NADPH-cytochrome c reductase was depressed [11], and the concentrations of cytochromes P-450 and b_5 were increased [12]. Table 1 shows the results from a typical experiment.

After thyroxine treatment for 7 days, 12α-hydroxylating activity in the liver microsomes fell to 49% of the control value (mean of 5 experiments). The yield of 12α-hydroxylated products was 16.1 ± 3.8% in the control preparations and 7.9 ± 1.7% in the preparations from the thyroxine-treated animals. In agreement with the finding of Phillips and Langdon [11], NADPH-cytochrome c reductase activity increased to about twice the control value. The concentrations of cytochromes P-450 and b_5 fell to about half the control values, showing that the effect of thyroxine treatment on both cytochromes is the opposite of the effect of thyroidectomy previously described by Suzuki et al. [12]. When the rats were killed at 2, 5 and 7 days after the beginning of a series of thyroxine injections, a fall in 12α-hydroxylating activity and in the concentrations of the two cytochromes, and a rise in NADPH-cytochrome c reductase activity, were detectable two days after the first injection (fig. 1).

These results suggest that the 12α-hydroxylation required for the formation of cholic acid from cholesterol is inhibited by thyroid hormone. Moreover, the enhancing effect of thyroidectomy suggests that the microsomal 12α-hydroxylase is partially inhibited or repressed under physiological conditions. Mitropoulos and Myant [13] and Berséus [14] have shown that thyroid hormone stimulates the oxidative cleavage of the side-chain of C_{27} sterols, an essential step in the formation of C_{24} bile acids from cholesterol. A combination of enhanced side-chain cleavage and diminished 12α-hydroxylation provides a possi-

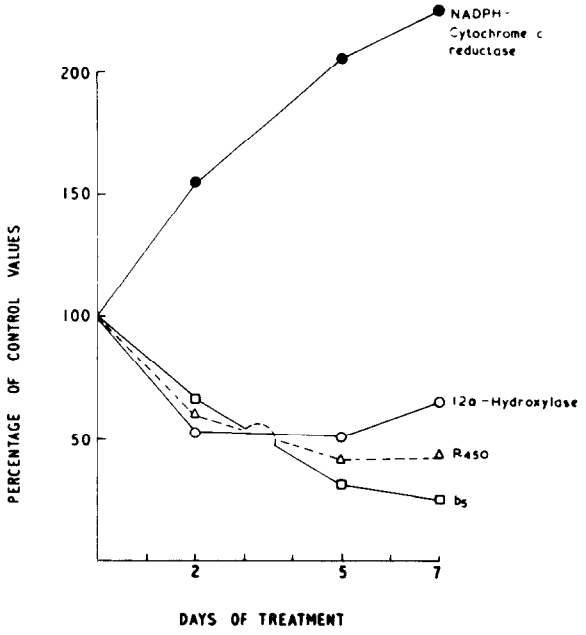

Fig.1. Effect of serial injections of thyroxine on the activities of 12α-hydroxylase and NADPH-cytochrome c reductase and on the concentrations of cytochromes P-450 and b_5 in rat-liver microsomes. In this experiment, the injections were begun 2, 5 and 7 days before all the rats were killed on the same day.

ble explanation for the changed pattern of bile acid production in thyroxine-treated rats.

There is evidence to suggest that NADPH-cytochrome c reductase may be a component of the electron-transfer chain concerned in microsomal 12α-hydroxylation [7]. The present work shows, however, that the activities of 12α-hydroxylase and NADPH-cytochrome c reductase change in opposite directions after thyroidectomy or thyroxine treatment. This makes it unlikely that the activity of NADPH-cytochrome c reductase is rate-limiting for 12α-hydroxylation in the test system we have used. The finding that the concentrations of P-450 and cytochrome b_5 change in parallel with 12α-hydroxylating activity might suggest that both cytochromes participate in this hydroxylation. However, we have shown elsewhere [7] that P-450 is not the terminal oxidase in the electron-transfer chain through which oxygen is activated for 12α-hydroxylation. There is at present no evidence concerning the possible role of cytochrome b_5 in 12α-hydroxylation.

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