ACTA CHEMICA SCANDINAVICA 19 (1965) 2131–2135

# On the Effect of Thyroid Hormones on the Oxidation of 5β-Cholestane-3α, 7α, 12α-triol

## Bile Acids and Steroids 157

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The oxidation of 5β-cholestane-3α,7α,12α-triol was studied in homogenates of liver from hyper-, eu-, and hypothyroid rats. Liver homogenates from hyperthyroid rats, treated with 3,5,3′-triiodo-L-thyronine for one week, showed an increase in capacity to oxidize this substrate as compared with normal rats, whereas liver homogenates from hypothyroid rats showed a decrease. After 3 weeks of treatment with thyroid hormone, the difference between hyperthyroid and normal rats with respect to oxidation of 5β-cholestane-3α,7α,12α-triol was increased. Addition of NADPH stimulated the oxidation to the same extent in both groups of homogenates.

The influence of thyroid hormones on cholesterol metabolism has been studied extensively in whole animals as well as in tissue preparations.[1] Evidence has been obtained to indicate that the thyroid hormones stimulate the degradation of cholesterol to bile acids. Strand [2] showed that the bile acid pool and the fecal excretion of bile acids are increased in the hyperthyroid rat as compared with the euthyroid rat. The main part of the enlarged bile acid pool consisted of chenodeoxycholic acid which was increased two- to three-fold above normal. The amount of cholic acid was unchanged giving a ratio between cholic acid and chenodeoxycholic acid of 3:2 in the hyperthyroid rat as compared with 4:1 in the euthyroid rat. The hypothyroid rat did not show any significant differences from the euthyroid rat with respect to pool size or bile acid composition. The effects of thyroid hormones have also been studied in bile fistula rats.[3–6] The total excretion of bile acids remained normal in the hyperthyroid rat but the ratio between cholic acid and chenodeoxycholic acid was 1:3 as compared with 3:1 in the euthyroid bile fistula rat. In the hypothyroid bile fistula rat there was a marked decrease in the total excretion of bile acids. The finding that the ratio between cholic acid and chenodeoxycholic acid is affected by treatment with thyroid hormones indicates a specific action of thyroid hormones on the biosynthesis of bile acids in the rat. The possibility

of such an effect of thyroid hormones is strengthened by the observation of the same changes in bile acid production in both intact rats and in bile fistula rats after administration of the D-isomer of 3,5,3′-triiodothyronine.[2,5,6]

The mechanisms of formation of cholic and chenodeoxycholic acids from cholesterol have been studied in detail.[7] In the biosynthesis of cholic acid the hydroxylations of the steroid nucleus and probably the other modifications of the nucleus, too, precede the oxidation of the side-chain. The oxidation of the $C_{27}$ side-chain is initiated by hydroxylation at position C-26 and in cholic acid formation the substrate for the 26-hydroxylase is likely 5$\beta$-cholestane-3$\alpha$,7$\alpha$,12$\alpha$-triol.[8–10] The 5$\beta$-cholestane-3$\alpha$,7$\alpha$,12$\alpha$,26-tetrol formed is transformed into 3$\alpha$,7$\alpha$,12$\alpha$-trihydroxy-5$\beta$-cholestanoic acid which in turn is oxidized to cholic acid and propionic acid.[8,9] Less is known of the exact mechanisms of chenodeoxycholic acid formation. One substrate for the 26-hydroxylase in this case is cholesterol itself which has been shown to be metabolized *in vitro* into cholest-5-ene-3$\beta$,26-diol.[11,12] However, several other compounds, including cholest-5-ene-3$\beta$,7$\alpha$-diol, cholest-4-en-7$\alpha$-ol-3-one, and 5$\beta$-cholestane-3$\alpha$,7$\alpha$-diol, are possible substrates for the 26-hydroxylase in chenodeoxycholic acid formation.[7,13–15] The degree of specificity of the enzymes catalyzing the oxidation of the $C_{27}$ side-chain is not known but the fact that a number of different $C_{27}$ neutral steroids is oxidized to $C_{24}$ bile acids might indicate a low specificity.

The change in ratio between cholic acid and chenodeoxycholic acid associated with administration of thyroid hormones to rats might be due to a stimulation of one or more of the enzymes concerned with the oxidation of the $C_{27}$ side-chain. If the enzyme(s) catalyzing the 26-hydroxylation has (have) a low degree of specificity, a stimulation of this reaction could lead to a proportionate increase in chenodeoxycholic acid formation; hydroxylation at C-26 of intermediates lacking a 12$\alpha$-hydroxyl group provides compounds that are converted into chenodeoxycholic acid.

In the present investigation, the oxidation of 5$\beta$-cholestane-3$\alpha$,7$\alpha$,12$\alpha$-triol was studied in homogenates of liver from hyper-, eu-, and hypothyroid rats. Liver homogenates from hyperthyroid rats showed an increase in capacity to oxidize this substrate and those from hypothyroid rats showed a decrease as compared with euthyroid rats.

After completion of this work a report by Mitropoulos and Myant [16] on oxidation of cholesterol in presence of mitochondria from liver of thyroxine-treated and normal rats appeared. These authors found an increase in production of labeled carbon dioxide and propionic acid from 26-$^{14}$C-cholesterol in presence of mitochondria from hyperthyroid rats as compared with mitochondria from normal rats, and they concluded that thyroxine stimulates a rate-limiting reaction leading to cleavage of the side-chain of cholesterol.

## EXPERIMENTAL

*Tritium-labeled 5β-cholestane-3α,7α,12α-triol.* This compound was prepared as described by Berséus, Danielsson, and Kallner.[17] Each incubation contained 0.1 $\mu$C of tritium-labeled 5$\beta$-cholestane-3$\alpha$,7$\alpha$,12$\alpha$-triol and material with four different specific activities, 0.5, 1, 2, and 4 $\mu$C per mg, was used.

*Animal experiments.* White male rats of the Sprague-Dawley strain weighing about 200 g were used. The rats were fed a commercial rat chow diet. Five groups of five animals

each were used. One group was made hypothyroid by giving drinking water containing 0.1 % propylthiouracil for 30 days. Two groups received 40 μg/kg body weight of 3,5,3′-triiodo-L-thyronine (Cytomel Sodium, Smith, Kline & French Laboratories, Philadelphia, Pa.) in daily subcutaneous injections for 7 and 21 days, respectively. The hormone was solubilized in slightly alkaline saline solution prior to injection. Two groups served as control and were treated with daily injections of the alkaline saline solution for 7 and 21 days, respectively. The oxygen consumption was measured for each rat every third day according to the method of Tomich and Woollett.[18] The hypothyroid rats showed a decrease in oxygen consumption (expressed as liter of oxygen/kg body weight/h) of 30—40 % and the hyperthyroid rats an increase of 50—70 % as compared with the average figure for the control animals.

*Preparation and analysis of the homogenates.* Homogenates, 33 % liver wet weight/volume, were prepared in 0.25 M sucrose containing 3.6 mg of nicotinamide per ml in a Potter-Elvehjem type homogenizer using a tight-fitting Teflon pestle. The homogenate was centrifuged for 15 min at 800 g. Each incubation contained 2 ml of whole homogenate and 1 ml of 0.1 M tris(hydroxymethyl) aminomethane-hydrochloric acid buffer, pH 7.6. The labeled 5β-cholestane-3α,7α,12α-triol was added in an emulsion stabilized with serum albumin.[19] All incubation mixtures were brought to a final volume of 4 ml by adding homogenizing medium and were incubated for 2 h at 37°. The incubations were terminated by addition of 4 volumes of ethanol. After filtration and evaporation of the ethanol, the aqueous solution was acidified with hydrochloric acid and was extracted with water-saturated butanol. The butanol extract was washed with water until neutral and the solvent was evaporated. The residue was chromatographed on a column of 4.5 g of Hostalene GW (Farbwerke Hoechst, W. Germany) using phase system III.[20] Three ml of stationary phase was used for 4.5 g of the support. The phase system used gives a separation into two fractions, one containing 5β-cholestane-3α,7α,12α,26-tetrol, 3α,7α,12α-trihydroxy-5β-cholestanoic acid, and cholic acid, the other containing unchanged 5β-cholestane-3α,7α,12α-triol. In order to separate the polar metabolites aliquots of the butanol extracts were subjected to thin layer chromatography on Kiselgel G (Merck, W. Germany) using benzene/dioxane/acetic acid, 10/5/1, as moving phase. The samples were chromatographed together with reference compounds as internal standards and were analyzed as earlier described.[17]

## RESULTS AND DISCUSSION

Fig. 1 summarizes the results of incubations of 5β-cholestane-3α,7α,12α-triol with homogenates of liver from hypothyroid, euthyroid, and hyperthyroid (treated with 3,5,3′-triiodo-L-thyronine for one week) rats. The extent of oxidation was measured with four different amounts of substrate, 25, 50, 100, and 200 μg, and as seen in Fig. 1 it was consistently greater in homogenates from hyperthyroid and lower in hypothyroid rats as compared with the control

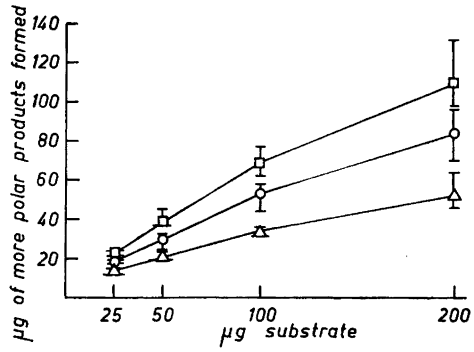


*Fig. 1.* Average and range of conversion of tritium-labeled 5β-cholestane-3α,7α,12α-triol into more polar products in liver homogenates from hyper- (□), eu- (○), and hypothyroid (△) rats (5 animals in each group).

group. The differences increased with increasing amounts of substrate. With the highest substrate concentration used, 200 μg, the average conversion of 5β-cholestane-3α,7α,12α-triol into more polar products was 110 μg in homogenates from hyperthyroid rats, 52 μg in those from hypothyroid rats, and 83 μg in the control group. The composition of the more polar products, consisting chiefly of 5β-cholestane-3α,7α,12α,26-tetrol, 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid, and cholic acid, was similar in all three groups of incubations and the average ratio between these products was 2:4:1.

The cofactor requirements for the oxidation of 5β-cholestane-3α,7α,12α-triol to cholic acid are not known in detail. In unpublished work from this laboratory it was found that the 26-hydroxylation of 5β-cholestane-3α,7α,12α-triol in a mitochondrial system from rat liver was greatly enhanced by addition of reduced pyridine nucleotides. Whitehouse, Staple, and Gurin [21] found that the formation of carbon dioxide from the side-chain of cholesterol in a similar system from rat liver was stimulated by addition of NADPH. It has been reported [22,23] that the levels of pyridine nucleotides, especially NADPH, are lower in livers from hyperthyroid rats than in those from euthyroid rats. On the other hand, McGuire and Tomkins [24] reported that the stimulation of a NADPH-dependent $\Delta^4$-3-ketosteroid 5α-reductase in rat liver occurring with thyroid hormone treatment for 3 days was due to an increased level of NADPH in the liver. However, after 2 to 3 weeks of treatment the stimulation was independent of NADPH level.

The results presented in Table 1 indicate that changes in the level of reduced pyridine nucleotides are not responsible for the differences in capacity to oxidize 5β-cholestane-3α,7α,12α-triol between hyper-, eu-, and hypothyroid rats. Liver homogenates from rats treated with 3,5,3′-triiodo-L-thyronine for 3 weeks, and from control rats were incubated with and without addition of NADPH. The stimulation obtained with NADPH was the same in both groups of animals. The extent of oxidation obtained in these experiments was lower than that in the experiments referred to in Fig. 1. The experiments reported in Table 1 were performed at a later date than those in Fig. 1 and with another shipment of animals.

The results of Mitropoulos and Myant [16] and of the present investigation indicate that thyroid hormones influence the biosynthesis of bile acids by

*Table 1.* Tritium-labeled 5β-cholestane-3α,7α,12α-triol, 100 μg, was incubated with liver homogenates from hyperthyroid rats (treated with 3,5,3′-triiodo-L-thyronine for 3 weeks) and from euthyroid rats. Four incubations were performed with each liver, two with addition of NADPH and two without addition.

| | Amount in μg of more polar products formed | |
|---|---|---|
| | No addition | 1.3 μmole of NADPH |
| Euthyroid rats (5 animals) | 31 (27—35) | 38 (34—40) |
| Hyperthyroid rats (5 animals) | 58 (53—63) | 86 (56—84) |

stimulation of the side-chain oxidation. It is possible that the change in ratio between cholic acid and chenodeoxycholic acid observed in hyperthyroid rats can be explained by this effect. However, thyroid hormones might also influence, at least to some extent, the 12$\alpha$-hydroxylation of a cholic acid precursor, and the effect of thyroid hormone treatment is presently being studied in an *in vitro* system capable of 12$\alpha$-hydroxylation of $C_{27}$ steroids.

*Acknowledgements.* I would like to thank Dr. Henry Danielsson for his interest and support during the course of this investigation. The skilful technical assistance of Miss Ingegerd Blomquist is gratefully acknowledged.

This work is part of investigations supported by the *National Heart Institute, U.S. Public Health Service* (Grant H-2842), the *Swedish Medical Research Council* (Project 13-X-218), and *Karolinska Institutets Reservationsanslag.*

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Received July 28, 1965.