Effects of Thyroxine on the Synthesis of Folate Coenzymes in Rat Liver

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1. The effects of thyroidectomy and of 'acute' and 'chronic' administration of thyroxine on the synthesis of folate coenzymes were studied by determining the liver contents of folate active derivatives and the enzymic activities involved in their biosynthesis. The effect of thyroxine on the same enzymes *in vitro* was also studied. 2. In thyroidectomized rats the liver contents of folate coenzymes did not change except for a slight decrease in the contents of 5-formyltetrahydrofolate and tetrahydrofolate compared with those in control rats. 3. In the same animals serine hydroxymethyltransferase and formyltetrahydrofolate synthetase activities decreased markedly. 4. The 'chronic' administration of thyroxine to thyroidectomized rats caused more evident variations in the liver contents of folate coenzymes and in particular a decrease in the contents of 5-formyltetrahydrofolate, tetrahydrofolate, 5(or 10)-formyl derivatives of tetrahydropteroylpolyglutamate and of 5(or 10)-formyl derivatives of pteroylpolyglutamate. 5. The enzymic activities did not show significant variations. 6. The 'acute' administration of thyroxine caused changes in the liver contents of some folate derivatives such as 10-formyl-dihydrofolate, 10-formylfolate, tetrahydrofolate and the 10-formyl derivative of dihydropteroylpolyglutamate. In these animals also the enzymic activities were unchanged. 7. No effect of thyroxine on enzymic activities *in vitro* was observed.

The administration of large doses of thyroxine as well as the feeding of thyroid powder or iodinated casein has been reported to have a remarkable effect on folate metabolism. Stokstad, Webb & Shah (1966) and Odell & Stokstad (1967) showed that rats fed on thyroid powder excreted abnormal amounts of formiminoglutamic acid, aminoimidazolecarboxamide and formate, which shows indirectly a decrease in the tissue storage of folate coenzymes involved in the metabolism of these intermediates. A similar effect was also observed in cases of thyrotoxicosis in man (Mohamed & Roberts, 1965).

Marchetti & Viviani (1959) and Viviani & Marchetti (1959) demonstrated more directly the influence of thyrotoxicosis in folate metabolism: rats treated with large doses of thyroxine showed a decrease of liver contents of citrovorum factor and a lower efficiency of liver in converting folate into its activated forms. Pasquali, Landi, Bovina & Marchetti (1970) observed, in the same experimental conditions, significant changes in the liver storage of most folate coenzymes. This effect might be ascribed to a decrease in the enzymic activities that are responsible for their synthesis.

Nevertheless the results obtained with toxic or pharmacological doses do not allow us to specify the exact mechanism of action of thyroid hormones on

folate metabolism and hence C₁ metabolism. It is well known that administration of different doses of thyroid hormones can cause biochemical changes in opposite directions.

This biphasic effect has been observed mostly with activities that depend on a balance between synthetic and degradative metabolic processes of the cell (Tata, 1964).

It is conceivable that when the hormone is present in the cells in very large amounts it acts differently to when it is present in much lower concentrations and thus masks the true physiological mode of action. We therefore studied the effects of small doses of thyroid hormones in folate metabolism.

In particular both 'chronic' and 'acute' effects of thyroxine on the content of various active derivatives of folate in the liver of thyroidectomized rats were studied. Also, the enzymic activity responsible for the overall conversion of folate into H₄folate,* H₄folate dehydrogenase (EC 1.5.1.3),

* Abbreviations: H₂folate, dihydrofolate; H₄folate, tetrahydrofolate; 5(or 10)-HCO-H₄folate, 5(or 10)-formyl-tetrahydrofolate; 5,10-CH₂-H₄folate, 5,10-methylene-tetrahydrofolate; 5,10-CH=H₄folate, methyldienetetrahydrofolate; 5(or 10)-HCO-H₄PteGlu_n, 5(or 10)-formyl derivatives of tetrahydropteroylpolyglutamic acid; 5(or 10)-HCO-Pte-Glu_n, 5(or 10)-formyl derivatives of pteroylpolyglutamic acid.

HCO-H₄folate synthetase (EC 6.3.4.3), 5,10-CH₂-H₄folate dehydrogenase (EC 1.5.1.5) and serine hydroxymethyltransferase (EC 2.1.2.1) were assayed.

The effect of various concentrations of thyroxine *in vitro* on the above-mentioned enzymic activities was also evaluated. Glucose 6-phosphate dehydrogenase (EC 1.1.1.49) activity, which is affected by thyroid hormones, was also determined in the liver of the animals in experiments to check both the whole ablation of the gland and the response to the administration of thyroxine (Tata *et al.* 1963).

MATERIALS AND METHODS

Materials. All chemicals, enzymes and coenzymes (except H₂folate and H₄folate) were obtained commercially. H₂folate was prepared by reduction of folate with dithionite (Futterman, 1957). H₄folate was prepared by catalytic hydrogenation of folate over platinum oxide in acetic acid (O'Dell, Vandenbelt, Bloom & Pfiffner, 1947).

Animals. Young male albino rats (Wistar) of body weight 60–70 g were divided into four groups. Group 1 was the control. The rats were killed 42 h after the last injection. Groups 2, 3 and 4 were thyroidectomized surgically under tribromoethanol anaesthesia and the thyroid region was carefully searched at autopsy to verify the total ablation of the thyroid gland. After 6 days, the rats of group 2 were given subcutaneous injections of alkaline 0.9% NaCl every fourth day for 6 weeks. The rats of group 3 were given subcutaneous injections of 40 µg of DL-thyroxine/100 g body wt. every fourth day for 6 weeks. The animals of group 4 were given a single injection of 60 µg of DL-thyroxine/100 g body wt. at the end of 6 weeks.

All thyroidectomized animals were fed throughout the experiments on a stock diet with no restrictions, and received calcium gluconate in their drinking water.

Analytical methods. To determine the folate coenzymes

liver acetone-dried powders were extracted in 1% (w/v) potassium ascorbate, pH 6.0, at 75°C for 30 min. The extracts were chromatographed on DEAE-cellulose-Hyflo Super Cel, and the eluted fractions were assayed for folate activities with *Pediococcus cerevisiae* A.T.C.C. 8081, *Streptococcus faecalis* A.T.C.C. 8043 and *Lactobacillus casei* A.T.C.C. 7469 (Marchetti, Pasquali & Landi, 1965). H₄folate dehydrogenase was assayed spectrophotometrically by measuring the decrease in *E*₃₄₀ caused by conversion of NADPH into NADP⁺ and of H₂folate to H₄folate (Mathews, Scrimgeour & Huennekens, 1963).

The overall conversion of folate into H₄folate was assayed by the method of Bratton & Marshall (1939).

The HCO-H₄folate synthetase and the 5,10-CH₂-H₄folate dehydrogenase were determined by measuring the 5,10-CH=H₄folate formed spectrophotometrically at 355 nm (Rabinowitz & Pricer, 1963; Scrimgeour & Huennekens, 1963).

Serine hydroxymethyltransferase was assayed by measuring colorimetrically both the free and the bound formaldehyde in 5,10-CH₂-H₄folate with the acetylacetone reagent (Scrimgeour & Huennekens, 1962).

To evaluate the effect of thyroid hormone *in vitro*, concentrations of DL-thyroxine ranging from 1 to 50 µM were added to the reaction mixtures with enzymic preparations from normal rats in the absence of substrates; the systems were preincubated for 30 min at 37°C and then the specific substrates were added.

Glucose 6-phosphate dehydrogenase was determined from the rate of reduction of NADP⁺, observed spectrophotometrically by the increase in *E*₃₄₀ (Glock & McLean, 1953).

Protein was determined by the colorimetric method of Lowry, Rosebrough, Farr & Randall (1951), with crystalline bovine plasma albumin (Sigma Chemical Co., St Louis, Mo., U.S.A.) as the standard.

RESULTS

The results in Table 1 on liver folate derivatives show only a slight decrease in the liver contents of 5-HCO-H₄folate (*P* < 0.01) and H₄folate (*P* < 0.01)

Table 1. *Effects of thyroidectomy and 'chronic' and 'acute' administration of thyroxine on the liver contents of various folate derivatives in the rat*

The methods of treatment of the rats and the methods for determining the folate coenzymes were as described in the Materials and Methods section. The values are expressed as means ± s.e.m. of ten observations on different animals. Significance of differences from values for normal animals: * *P* < 0.05; † *P* < 0.01; ‡ *P* < 0.001.

Compounds	Liver folate derivatives (µg/g wet wt. of tissue)			
	Group 1 (Normal rats)	Group 2 (Thyroidectomized rats)	Group 3 (Thyroidectomized rats with 'chronic' thyroxine treatment)	Group 4 (Thyroidectomized rats with 'acute' thyroxine treatment)
10-HCO-H ₄ folate	1.33 ± 0.09	1.37 ± 0.12	1.05 ± 0.12	1.76 ± 0.15
10-HCO-H ₂ folate and 10-HCO-folate	2.11 ± 0.17	2.06 ± 0.19	1.94 ± 1.70	1.08 ± 0.08†
5-HCO-H ₄ folate	0.62 ± 0.05	0.40 ± 0.03†	0.23 ± 0.03‡	0.69 ± 0.05
10-HCO-H ₄ PteGlu _n	9.46 ± 0.81	8.60 ± 0.92	5.84 ± 0.40†	4.30 ± 0.35‡
H ₄ folate	0.46 ± 0.04	0.32 ± 0.02†	0.16 ± 0.01‡	0.28 ± 0.05*
10-HCO-PteGlu _n	0.64 ± 0.07	0.69 ± 0.05	0.21 ± 0.01‡	0.55 ± 0.04
5-HCO-H ₄ PteGlu _n	0.43 ± 0.03	0.45 ± 0.03	0.10 ± 0.01‡	0.52 ± 0.04
5-HCO-PteGlu _n	0.29 ± 0.02	0.23 ± 0.02	0.16 ± 0.02‡	0.30 ± 0.02

in thyroidectomized rats (group 2) as compared with control animals (group 1). A more evident decrease in the content of various coenzymes, especially 5-HCO-H₄folate ($P < 0.001$), H₄folate ($P < 0.001$), 5(or 10)-HCO-H₄PteGlu_n ($P < 0.001$) and 5(or 10)-HCO-PteGlu_n ($P < 0.001$), was observed in thyroidectomized and 'chronically' thyroxine treated rats (group 3).

Thyroidectomized rats injected with a single dose of thyroxine (group 4) showed a decrease of 10-HCO-H₂folate and 10-HCOfolate ($P < 0.001$), 10-HCO-H₄PteGlu_n ($P < 0.001$) and H₄folate ($P < 0.05$), whereas the liver contents of the other coenzymes were not modified.

The results in Table 2 show that liver glucose 6-phosphate dehydrogenase activity decreases in thyroidectomized rats ($P < 0.001$). Chronic treatment with thyroxine markedly enhances this enzyme activity ($P < 0.001$), whereas acute treatment does not modify significantly the glucose

6-phosphate dehydrogenase activity measured after 42h.

The activities of H₄folate dehydrogenase and of the enzymic system that converts folate into H₄folate do not appear to be significantly modified in thyroidectomized rats. Also, neither 'chronic' nor 'acute' administration of thyroxine affects these activities.

The results on the enzymes responsible for the synthesis and interconversion of C₁ derivatives of H₄folate (Table 3) show that serine hydroxymethyltransferase and HCO-H₄folate synthetase activities were significantly lower in the liver of thyroidectomized rats compared with the controls ($P < 0.05$). On the other hand, 5,10-CH₂-H₄folate dehydrogenase activity did not show significant variation.

In thyroidectomized rats and 'chronically' thyroxine-treated rats no variation was seen in the three enzymic activities.

Table 2. *Effects of thyroidectomy and 'chronic' and 'acute' administration of thyroxine on liver H₄folate dehydrogenase and the enzymic activity that converts folate into H₄folate*

The methods of treatment of the rats and the methods for measuring the enzymic activities were as described in the Materials and Methods section. The values are expressed as means $\pm$ s.e.m. of ten observations on different animals.

Group	Experimental animals	Glucose 6-phosphate dehydrogenase (ΔE_{340} /min per mg of protein)	H ₄ folate dehydrogenase (nmol of H ₂ folate reduced/min per mg of protein)	Enzymic system converting folate into H ₄ folate (nmol of H ₄ folate formed/40 min per mg of protein)
1	Normal	0.042 $\pm$ 0.003	5.01 $\pm$ 0.72	1.24 $\pm$ 0.04
2	Thyroidectomized	0.025 $\pm$ 0.002	4.40 $\pm$ 0.13	1.06 $\pm$ 0.09
3	Thyroidectomized, with 'chronic' thyroxine treatment	0.145 $\pm$ 0.011	4.35 $\pm$ 0.24	1.26 $\pm$ 0.05
4	Thyroidectomized, with 'acute' thyroxine treatment	0.035 $\pm$ 0.003	4.38 $\pm$ 0.12	1.25 $\pm$ 0.04

Table 3. *Effects of thyroidectomy and 'chronic' and 'acute' administration of thyroxine on the liver serine hydroxymethyltransferase, HCO-H₄folate synthetase and 5,10-CH₂-H₄folate dehydrogenase*

The methods of treatment of the rats and the methods for measuring the enzymic activities were as described in the Materials and Methods section. The values are expressed as means $\pm$ s.e.m. of ten observations on different animals. Significance of differences from values for normal animals: * $P < 0.05$.

Group	Experimental animals	Serine hydroxymethyltransferase (nmol of HCHO utilized/20 min per mg of protein)	HCO-H ₄ folate synthetase (nmol of 5,10-CH=H ₄ folate formed/20 min per mg of protein)	5,10-CH ₂ -H ₄ folate dehydrogenase (nmol of 5,10-CH=H ₄ folate formed/20 min per mg of protein)
1	Normal	1237 $\pm$ 164	1460 $\pm$ 120	370 $\pm$ 11
2	Thyroidectomized	833 $\pm$ 71*	1052 $\pm$ 105*	349 $\pm$ 18
3	Thyroidectomized, with 'chronic' thyroxine treatment	980 $\pm$ 87	1610 $\pm$ 360	396 $\pm$ 11
4	Thyroidectomized, with 'acute' thyroxine treatment	1172 $\pm$ 224	1865 $\pm$ 224	442 $\pm$ 39*

Table 4. *Effects of thyroxine in vitro on H₄folate dehydrogenase, the enzymic system that converts folate into H₄folate, serine hydroxymethyltransferase, HCO-H₄folate synthetase and 5,10-CH₂-H₄folate dehydrogenase*

Thyroxine was added to the reaction mixtures with enzymic preparations from normal rats in the absence of substrates. The systems were preincubated for 30 min at 37°C and after addition of the specific substrates the various enzymic activities were measured as described in the Materials and Methods section. The values are expressed as means $\pm$ s.e.m. of six observations on different animals.

Enzyme	Thyroxine added to the reaction mixtures	...	—	1 μ M	5 μ M	50 μ M
H ₄ folate dehydrogenase (nmol of H ₂ folate reduced/min per/mg of protein)		4.90 $\pm$ 0.35		5.01 $\pm$ 0.38	4.90 $\pm$ 0.42	4.65 $\pm$ 0.32
Enzymic system converting folate into H ₄ folate (nmol H ₄ folate formed/40 min per mg of protein)		1.61 $\pm$ 0.09		1.51 $\pm$ 0.08	1.58 $\pm$ 0.04	1.49 $\pm$ 0.06
Serine hydroxymethyltransferase (nmol of HCHO utilized/20 min per mg of protein)		1380 $\pm$ 165		1086 $\pm$ 130	1238 $\pm$ 148	1274 $\pm$ 153
HCO-H ₄ folate synthetase (nmol of 5,10-CH=H ₄ -folate formed/20 min per mg of protein)		1178 $\pm$ 153		201 $\pm$ 172	1004 $\pm$ 130	1210 $\pm$ 157
5,10-CH ₂ -H ₄ folate dehydrogenase (nmol of 5,10-CH=H ₄ folate formed/20 min per mg of protein)		332 $\pm$ 25		347 $\pm$ 30	335 $\pm$ 28	342 $\pm$ 35

In rats thyroidectomized and injected with a single dose of thyroxine, 5,10-CH₂-H₄folate dehydrogenase activity increased significantly ($P < 0.05$) whereas those of serine hydroxymethyltransferase and HCO-H₄folate synthetase were not changed.

Lastly, as shown in Table 4, the addition of thyroxine at concentrations ranging from 1 to 50 μ M in the reaction mixtures does not modify significantly the examined enzymic activities.

DISCUSSION

The values for the liver contents of folate coenzymes and the enzymic activities responsible for their synthesis in thyroidectomized rats and thyroxine-treated rats help to explain the mechanism of action of thyroid hormone on folate metabolism. First, it was shown that thyroidectomy does not influence the reductive steps that convert folate into H₄folate. In thyroidectomized rats the activities of H₄folate dehydrogenase and the enzymic system that converts folate into H₄folate are the same as in normal rats. On the other hand thyroidectomy probably influences the processes that give rise to C₁ derivatives of H₄folate. Serine hydroxymethyltransferase and HCO-H₄folate synthetase, which catalyse respectively the transfer of the β -carbon of serine and of formic acid to the H₄folate, are markedly decreased in activity.

Although the liver of thyroidectomized rats synthesized less effectively the active forms transferring the C₁ unit, the liver content of these enzymes was not changed significantly. This could be ascribed to a smaller utilization of the C₁ units, since most of the metabolic processes in which folate coenzymes are involved appear to be greatly depressed in this experimental condition.

'Chronic' administration of thyroxine to thyroidectomized rats tends to normalize enzymic activities involved in the biosynthesis of these coenzymic derivatives, but it also causes a decrease in liver contents of the coenzymes.

In this case the decreased storage of folate coenzymes in the liver of the animals receiving small doses of thyroxine could be caused only by an increased utilization and not by a decreased biosynthesis as occurs in thyrotoxicosis induced by high doses of hormone (Pasquali *et al.* 1970).

Also the 'acute' administration of thyroxine, which is able to restore the enzymic activities depressed by thyroidectomy and to increase some of them, does not cause any increase in the storage of coenzymes.

The results could clearly demonstrate that thyroid hormone can affect folate metabolism and especially the synthesis of its coenzymic forms. Nevertheless this could be a secondary effect; i.e. the synthesis could depend on the influence of the hormone on many metabolic processes in which these coenzymes are involved.

This hypothesis is in agreement with the observation that the addition of thyroxine *in vitro* does not modify the enzymic activities involved in the synthesis of folate coenzymes. Therefore the lack of this effect *in vitro* excludes a direct hormone-enzyme interaction.

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