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N-METHYL OXIDATION IN LIVER MITOCHONDRIA OF TRIIODOTHYRONINE-TREATED AND THYROIDECTOMIZED RATS

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SUMMARY

The levels of sarcosine dehydrogenase and acid-nonextractable flavin in the inner matrix of mitochondria of rat liver are decreased in animals treated with triiodothyronine and are elevated in the mitochondria obtained from thyroidectomized animals. Administration of triiodothyronine does not affect the electron-transfer flavoprotein associated with the sarcosine dehydrogenase or the relative amounts of soluble and membrane-bound proteins of the mitochondria. In phosphate-washed mitochondria from either the controls or the triiodothyronine-treated rats, the O_2 uptake equals the total of the [^{14}C]formaldehyde and [β-^{14}C]serine isolated as reaction products of the sarcosine-[^{14}C]methyl group. In contrast to its restraint of sarcosine or choline oxidation in preparations capable of oxidative phosphorylation, ADP does not inhibit the oxidation of these substrates in mitochondria of rats given triiodothyronine.

INTRODUCTION

Mitochondrial flavoenzymes which contain peptide-linked, or acid-nonextractable, flavins as prosthetic groups include succinate dehydrogenase [1, 2], monoamine oxidase [3–5], and sarcosine dehydrogenase [6, 7]. Succinate dehydrogenase is located in the inner membrane [8–10] while the monoamine oxidase is associated with the outer membrane [10]. The sarcosine dehydrogenase, on the other hand, is not membrane-bound and is found in the inner matrix of the mitochondrion [11].

Earlier studies had demonstrated that administration of dessicated thyroid to rats can result in a 10–20 fold increase in the α-glycerophosphate dehydrogenase activity of the liver mitochondria [12]. The effects on the succinate and choline dehydrogenases were not considered significant. Subsequent analyses carried out in this laboratory have shown that the sarcosine dehydrogenase activity is decreased in rats given triiodothyronine and is increased in thyroidectomized animals. The purposes of the studies reported here were to explore further the effects of the thyroid

Abbreviations: DCIP; 2,6-dichlorophenolindophenol, PMS; phenazine methosulfate.

hormone on flavoenzymes involved in oxidative metabolism of one-carbon compounds and also to determine the levels of covalently-bound flavins in both the matrix and membrane fractions of the liver mitochondria of animals treated with the hormone.

METHODS AND MATERIALS

Mitochondria of rat liver were isolated in 0.25 M sucrose or 0.15 M KCl, and respiration and dehydrogenase activities were measured as described previously [13, 14]. The composition of the reaction mixtures are given in the legends of the tables. Analyses of flavins, protein, [^{14}C]formaldehyde, and [β-^{14}C]serine were carried out as in earlier studies [7, 15, 16].

Sprague-Dawley rats, averaging 200 g, were given four intraperitoneal injections of 100 μg of triiodothyronine per 100 g of body weight at 24 h intervals and were sacrificed 24 h after the last injection. The control animals were treated in the same manner with comparable volumes of 0.9 % saline. Thyroidectomized rats were supplied by Charles River Breeding Labs.

Sarcosine, free base, was obtained from Fluka AB and was recrystallized from ethanol–water. [*Me*-^{14}C]sarcosine was purchased from New England Nuclear. Triiodothyronine was from Sigma Chemical. All of the other reagents were commercial products of highest quality.

RESULTS AND DISCUSSION

Oxidation of sarcosine, choline, and α-glycerophosphate in liver mitochondria of triiodothyronine-treated rats

Typical analyses showing the differences in initial rates of O_2 uptake with sarcosine, succinate and α-glycerophosphate in intact mitochondria from control and triiodothyronine-treated rats are summarized in Table I. Confirming earlier results of others [12], the oxidation of α-glycerophosphate was found to be stimulated 5–20 fold, while the enhancement of the activities with succinate (and choline) was less pronounced. The specific activity of the sarcosine oxidase, in contrast, is decreased markedly in the mitochondria from the triiodothyronine-treated animals. The same

TABLE I

OXIDATION OF SARCOSINE, SUCCINATE, AND α-GLYCEROPHOSPHATE IN MITOCHONDRIA OF TRIIODOTHYRONINE-TREATED RATS

Reaction mixtures contained: mitochondria (16.1 mg protein from controls; 17.0 mg protein from triiodothyronine-treated animals) in 0.075 M potassium phosphate–0.0001 M Mg^{2+} (1.0 ml); substrate, 10 μmoles (0.05 ml); and phosphate –Mg buffer to a final volume of 2.6 ml. Temp., 30 °C.

Substrate	O_2 uptake (μatoms/10 min per mg protein)	
	Control	Triiodothyronine-treated
Sarcosine	0.047	0.028
Succinate	0.80	0.80
α-Glycerophosphate	0.032	0.19

results are obtained when the sarcosine dehydrogenase in the matrix fraction of the mitochondria is assayed by 2,6-dichlorophenolindophenol (DCIP) reduction either in the absence or presence of phenazine methosulfate (PMS). Hence it can be concluded that triiodothyronine treatment affects the dehydrogenase rather than the electron-transfer flavoprotein component [17] of the sarcosine demethylation system. In control experiments, the addition of triiodothyronine to normal mitochondria at a level of 4 μg per mg of protein did not alter the rate of sarcosine oxidation.

Under the same experimental conditions described in Table I, it was found that the oxidative demethylation of dimethylglycine is also depressed, and to the same degree as with sarcosine, in liver mitochondria of triiodothyronine-treated rats.

Applying methods for quantitatively separating the matrix and membranous fractions of liver mitochondria [11], it was of interest to find in the current investigation that the relative amounts of soluble and membrane-bound protein are nearly identical in the mitochondria of the untreated and triiodothyronine-treated animals. These results show that hormone treatment does not alter membrane integrity to a degree which leads to a differential loss of matrix enzymes during the isolation procedures.

Quantitative isolation of oxidation products of the N–CH_3 group of sarcosine in mitochondria of triiodothyronine-treated rats

To determine whether triiodothyronine treatment affects the level as well as the rate of oxidation of the N–CH_3 group of sarcosine, the reaction products were assayed quantitatively as described in Table II. It will be noted that the O_2 uptake equalled the total of the formaldehyde and serine isolated in either the control or triiodothyronine-treated preparations. Moreover, even though the rate of oxidation of sarcosine in the mitochondria from the animals administered hormone was less than 40% of the rate observed in the controls, the relative amount of serine produced was not depressed. These findings suggest that triiodothyronine does not significantly affect the enzyme system(s) involved in the oxidation or transfer of one carbon units with oxidation levels equal to or higher than that of formaldehyde.

TABLE II

QUANTITATIVE ISOLATION OF OXIDATION PRODUCTS OF THE N-METHYL GROUP IN MITOCHONDRIA OF TRIIODOTHYRONINE-TREATED RATS

Reaction mixtures contained: mitochondria (19.1 mg protein from controls; 13.1 mg protein from triiodothyronine-treated animals) in 0.6 ml 0.075 M potasssium phosphate–0.0001 M Mg^{2+}; [*Me*-^{14}C]sarcosine, 10 μmoles (0.1 ml), 20 950 cpm per μmole (corrected); phosphate–Mg buffer to a final volume of 2.6 ml. O_2 uptake was measured for 60 min. $H^{14}CHO$ and [β-^{14}C]serine were isolated as described elsewhere [16], and were analyzed in a Beckman scintillation counter, Model LS-133, employing 15 ml portions of Aquasol (New England Nuclear) scintillation fluid. Temp., 30 °C.

	Control	Triiodothyronine treated
Oxygen uptake (μatoms)	4.43	1.65
$H^{14}CHO$ (μmoles)	3.97	1.32
[β-^{14}C]serine (μmoles)	0.58	0.34

Sarcosine and choline oxidation in mitochondria of triiodothyronine-treated rats in the presence of ADP

As in the case of choline [18], the rate of oxidation of sarcosine in phosphorylating mitochondria is restrained in the presence of ADP [14]. The effect is not observed when uncoupling agents such as dinitrophenol or pentachlorothiophenol are added to the system [14]. It has also been shown previously that phosphorylation efficiency can be decreased in liver mitochondria of thyroid-fed rats [19]. Consistent with these findings, the present studies have demonstrated that ADP does not significantly influence the rate of oxidation of either sarcosine or choline in mitochondria from triiodothyronine-treated rats (Table III). Irrespective of the mechanism by which ADP may affect the movement of sarcosine and choline across the mitochondrial membranes [14, 20] or the efflux of betaine [21], these results provide additional evidence that the restraining influence of ADP is sensitive to the "energy state" of the mitochondria.

TABLE III

SARCOSINE AND CHOLINE OXIDATION IN LIVER MITOCHONDRIA OF TRIIODOTHYRONINE-TREATED RATS IN PRESENCE OF ADP

Reaction mixtures contained: mitochondria (16.3 mg protein from controls; 14.2 mg protein from triiodothyronine-treated rats) in 1.2 ml 0.15 M KCl; sarcosine or choline, 10 μmoles (0.1 ml); ADP (5 μmoles), 0.1 ml; potassium pyrophosphate, 90 μmoles (1.2 ml), pH 7.5. Temp., 30 °C.

Substrate	O_2 uptake (μatoms/20 min/mg protein)			
	Control		Triiodothyronine-treated	
	−ADP	+ADP	−ADP	+ADP
Sarcosine	0.110	0.026	0.027	0.025
Choline	0.225	0.082	0.194	0.179

Acid-extractable and acid-nonextractable flavins of liver mitochondrial fractions from triiodothyronine-treated rats

The data in Fig. 1 show the flavin compositions of those fractions of the mitochondria containing sarcosine dehydrogenase and α-glycerophosphate dehydrogenase. The analyses of acid-nonextractable flavins, carried out as described previously [7, 14], employed the established procedure of measuring differences in fluorescence at pH 3.0 and 7.0 [22, 23]. This method is quantitative for acid-nonextractable flavins of the type such as in succinate dehydrogenase in which the 8 α-methyl group on the isoalloxazine is linked via a histidyl residue to the peptide chain and in which there is no neighboring tyrosyl group. The same analysis is not suitable for determining flavins in which the peptide is linked to the riboflavin as a thioether of cysteine [24] or by the ε-N of a lysine [25]. Evidence to date suggests that the peptide flavin of sarcosine dehydrogenase of liver mitochondria is of the histidyl type as in succinate dehydrogenase [7].

The average level of acid-nonextractable flavin in the matrix compartment of rat liver mitochondria is in the range of 0.12–0.18 μmole/g of soluble protein [15]. Consonant with the decrease in specific activity of the sarcosine dehydrogenase

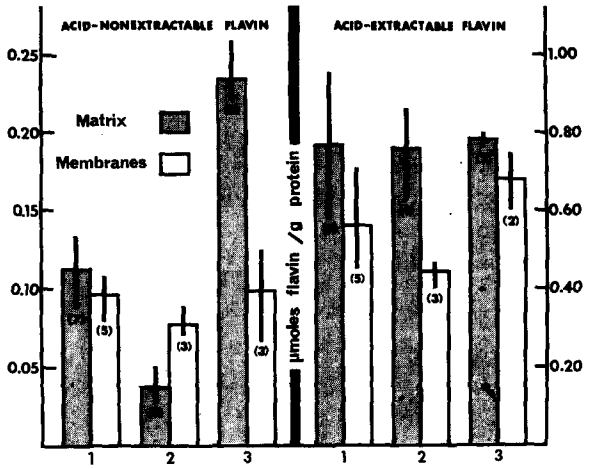

Fig. 1. Acid-extractable and acid-nonextractable flavins of liver mitochondria of triiodothyronine-treated and thyroidectomized rats. The soluble (matrix) and membranous fractions were isolated from mitochondria of (1) untreated, (2) triiodothyronine-treated, and (3) thyroidectomized rats, and the flavins were analyzed as described under Methods and Materials. The number of animals used in each group and the ranges of flavin/protein ratios are indicated at the tops of the bars.

following administration of triiodothyronine, the quantity of acid-nonextractable flavin found in the inner matrix of the mitochondria from the triiodothyronine-treated animals was lowered to almost exactly the same degree.

One-carbon metabolism in mitochondria from thyroidectomized rats

Compatible with the depression of N-methyl oxidation in mitochondria from rats treated with triiodothyronine, the opposite effect was observed in similar mitochondrial preparations from thyroidectomized animals. As is evident from the

TABLE IV

SARCOSINE OXIDATION IN LIVER MITOCHONDRIA OF THYROIDECTOMIZED RATS

Reaction mixtures for the oxidase analyses contained: mitochondria (30.7 mg protein from thyroidetomized animals; 27.1 mg protein from controls), in 1.0 ml of 0.075 M potassium phosphate – 0.0001 M $MgCl_2$, pH 7.5; substrates, 10 μmoles (0.1 ml); and 0.075 M phosphate – 0.0001 M Mg buffer to a final volume of 2.6 ml. The dehydrogenase assays were carried out with sonically irradiated mitochondria: 1.9 mg protein from controls, and 3.2 mg protein from thyroidectomized animals, in a final volume of 3.0 ml. Temp., 30 °C.

Substrate	Oxidase activity (μatoms oxygen/20 min per mg protein)		Dehydrogenase activity (nmoles DCIP reduced/1.5 min per mg protein)	
	Control	Thyroidectomized	Control	Thyroidectomized
Sarcosine	0.052	0.142	24	65
Choline	0.288	0.305	—	—
Succinate	0.576	0.530	—	—
α-Glycerophosphate	0.042	0.011	35	7

data of Table IV, the rate of sarcosine oxidation measured either as the oxidase or the dehydrogenase activity is higher in the mitochondria from the thyroidectomized rats than in the controls. Predictably, the oxidation of α-glycerophosphate is markedly lowered by thyroidectomy. The rates of dehydrogenation of choline and succinate, on the other hand, are not significantly different from the controls. As in all previous cases in which the level of sarcosine dehydrogenase in the mitochondrial matrix has been altered, the degree to which the concentration of peptide-bound flavin is elevated in the matrix fraction of the mitochondria from the thyroidectomized animals is that expected from the observed increase in dehydrogenase activity (Fig. 1).

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