INFLUENCE OF L-THYROXINE ON KYNURENINE 3-HYDROXYLASE, MONOAMINE OXIDASE, AND ROTENONE-INSENSITIVE NADH-CYTOCHROME C REDUCTASE IN MITOCHONDRIAL OUTER MEMBRANE*

Hiroshi Okamoto

Department of Medical Chemistry
Kyoto University Faculty of Medicine
Kyoto 606, Japan

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SUMMARY

Subcutaneous administration of L-thyroxine to rats at a dose of 75 μg/100 g of body weight/day for 12 days resulted in a decrease in the activities of kynurenine 3-hydroxylase, monoamine oxidase, and rotenone-insensitive NADH-cytochrome c reductase in the outer membrane of liver mitochondria to about 50% of control values. L-Thyroxine in concentrations of 1 and 5 μg/ml had no effect on these enzyme activities *in vitro*. In contrast, certain enzymes localized in the inner mitochondrial membrane showed an increase in activity by the L-thyroxine administration.

Membrane-specific responses of intramitochondrial enzymes to thyroid hormone are discussed.

Thyroid hormones accelerate cellular reactions in practically every organ and tissue of the body. The ability of the hormones to accelerate oxygen consumption of excised tissues from hyperthyroid animals (1) is accompanied by increased level of mitochondrial enzymic systems that have been studied (2-8). Recently, studies on the intramitochondrial enzyme localization have been carried out and provided evidence that the outer and the inner membrane of liver mitochondria are completely different in enzymic make up. The outer membrane contains at least the following three

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enzymes; kynurenine 3-hydroxylase (9-12), monoamine oxidase (13,14), and rotenone-insensitive NADH-cytochrome c reductase (15), whereas the inner membrane-matrix fraction contains mainly respiratory chain and phosphorylating enzymes and citric acid cycle enzymes (14,16).

In order to see if any control mechanism could take place between the outer and the inner mitochondrial membranes, I have undertaken the study of the influence of thyroid hormone on the enzymes of the two different mitochondrial membranes. Data to be presented in this paper indicate that following the administration of L-thyroxine in rats, the activities of the three outer membrane enzymes, kynurenine 3-hydroxylase, monoamine oxidase, and rotenone-insensitive NADH-cytochrome c reductase are decreased to about 50% of the original values. On the other hand, the enzymes localized in the inner membrane show increases in activity.

MATERIALS and METHODS

Male Wistar rats weighing from 180 g to 220 g which were maintained on commercial complete rat diet (laboratory chow) were used. L-Thyroxine (sodium salt, Sigma Chemical Company) was dissolved in NaOH (pH 8.5), diluted with 0.85% NaCl and administered subcutaneously.

Rat liver mitochondria which were prepared in cold 0.21 M mannitol containing 0.07 M sucrose and 0.1 mM EDTA (9-12) principally by the method of De Duve et al. (17) were washed six times with the mannitol solution and the mitochondrial outer membrane was prepared as previously described (18).

Kynurenine 3-hydroxylase (9-12), monoamine oxidase (13), rotenone-insensitive NADH-cytochrome c reductase (15), glycerophosphate dehydrogenase (19), succinate-cytochrome c reductase

(15), glutamate dehydrogenase (14), malate dehydrogenase (13), and kynurenine aminotransferase (18) were assayed as previously described. Protein was determined by the method of Lowry et al.(20).

RESULTS

The activities of various enzymes in liver mitochondria isolated from normal and L-thyroxine-administered rats are summarized in Table I. The activities of kynurenine 3-hydroxylase, monoamine oxidase, and rotenone-insensitive NADH-cytochrome c reductase of mitochondria from L-thyroxine-treated rats were all de-

TABLE I

Effect of L-thyroxine on intramitochondrial enzymes

Enzymes	Liver Mitochondria from		
	Group A Sp.Act.(%)	Group B Sp.Act.(%)	Group C Sp.Act.(%)
Outer Membrane Enzymes;			
Kynurenine 3-hydroxylase	3.2(100)	1.6 (50)	3.5(109)
Monoamine oxidase	15.0(100)	6.8 (45)	13.5 (90)
Rotenone-insensitive NADH-cytochrome c reductase	170 (100)	104 (61)	196 (115)
Inner Membrane-Matrix Enzymes;			
Glycerophosphate dehydrogenase	10 (100)	150 (1500)	37 (370)
Succinate-cytochrome c reductase	210 (100)	378 (180)	242 (115)
Glutamate dehydrogenase	90 (100)	126 (140)	———
Malate dehydrogenase	1700 (100)	2040 (120)	———
Kynurenine aminotransferase	4.4(100)	5.1(116)	———

Group A consists of normal rats fed laboratory chow; Group B, normal rats given 75 µg of L-thyroxine-Na per 100 g of body weight per day for 12 days; Group C, normal rats given L-thyroxine-Na (75 µg/100 g of body weight/day) for 12 days and killed 7 days later. Specific activity (Sp. Act.) represents the mean of 6 rats and is expressed as mµmoles/min/mg of mitochondrial protein. Within each specific activity, the results deviated from the mean by less than 25%. The numbers in parenthesis are expressed as a percentage of the activity with Group A.

creased to about 50% of the control values and the decreased enzyme activities were completely recovered by withdrawing the hormone. Maximum depressions of the enzyme activities occurred after 7-12 days of treatment for kynurenine 3-hydroxylase and rotenone-insensitive NADH-cytochrome c reductase, and after 10-14 days for monoamine oxidase. Further administration of L-thyroxine caused no further decrease in activity.

In contrast, activities of glycerophosphate dehydrogenase, succinate-cytochrome c reductase, glutamate dehydrogenase, malate dehydrogenase, and kynurenine aminotransferase, which are localized in the inner mitochondrial membrane (14,18,21), were increased 15- to 1.2-fold by the L-thyroxine administration.

Table II shows the results of the assays of kynurenine 3-hydroxylase, monoamine oxidase, and rotenone-insensitive NADH-cytochrome c reductase, which were carried out with the outer membranes isolated from liver mitochondria of normal and L-thyroxine-

TABLE II

Activities of kynurenine 3-hydroxylase, monoamine oxidase, and rotenone-insensitive NADH-cytochrome c reductase in the mitochondrial outer membranes.

Enzymes	Outer Membrane from			
	Group A		Group B	
	Sp. Act.	(%)	Sp. Act.	(%)
Kynurenine 3-hydroxylase	25	(100)	14.2	(57)
Monoamine oxidase	105	(100)	56	(53)
Rotenone-insensitive NADH-cytochrome c reductase	1100	(100)	726	(66)

Outer membrane was isolated from the mitochondria of Group A or Group B of Table I. Specific activity (Sp. Act.) is expressed as mμmoles/min/mg of the outer membrane protein. The numbers in parenthesis are expressed as a percentage of the activity with Group A.

TABLE III

The *in vitro* effect of L-thyroxine on the activities of kynurenine 3-hydroxylase, monoamine oxidase, and rotenone-insensitive NADH-cytochrome c reductase in rat liver mitochondria.

Enzymes	Final L-Thyroxine Concentration (μg/ml)		
	0	1	5
	mμmoles/min/mg protein		
Kynurenine 3-hydroxylase	3.3	3.4	3.1
Monoamine oxidase	15.0	16.0	15.2
Rotenone-insensitive NADH-cytochrome c reductase	166	176	177

Enzyme activity assayed in the presence (1 μg/ml and 5 μg/ml) and absence of L-thyroxine-Na. Liver mitochondria, which were prepared from normal rats, were used as an enzyme sample.

treated rats. It was confirmed that these enzyme activities in the outer membrane isolated from L-thyroxine-treated rats were decreased to about 50% of the control values. That the *in vivo* effects of L-thyroxine depicted in Table I and II were not due to some direct effect of L-thyroxine on the enzymes has been ruled out by the *in vitro* experiments (Table III) where L-thyroxine concentrations up to 5 μg/ml did not affect activity.

DISCUSSION

The present finding suggests that the topographically different intramitochondrial enzymes are differently affected by thyroid hormone. As a result of the daily administration of L-thyroxine, the activities of kynurenine 3-hydroxylase, monoamine oxidase, and rotenone-insensitive NADH-cytochrome c reductase localized in the outer membrane were decreased, whereas the activities of the enzymes localized in the inner membrane were increased. Zile and Lardy (22) showed in 1959 that thyroid-fed rats possessed lower

monoamine oxidase activity of liver mitochondria than normal rats. In the present study, we confirmed that the monoamine oxidase activity in the outer membrane isolated from L-thyroxine-administered rats was decreased to about 50% of the control value. It has been already reported that the increase in activity of mitochondrial enzymes, such as glycerophosphate dehydrogenase and succinate-cytochrome c reductase, occurred as a result of thyroid hormone administration (3-8) and that thyroid hormone induced the de novo synthesis of glycerophosphate dehydrogenase (5-8).

The mechanism of suppression of activity of mitochondrial outer membrane enzymes (i.e. kynurenine 3-hydroxylase, monoamine oxidase, and rotenone-insensitive NADH-cytochrome c reductase) in rats receiving L-thyroxine is uncertain. Although the possibility that the decrease in the enzyme activity is ascribed to some structural alteration of the outer membrane as a whole is not ruled out, a reasonable explanation is that L-thyroxine reflected an inhibitory action on the synthesis of these outer membrane enzymes. In fact, mitochondrial outer membrane preparations isolated from normal and L-thyroxine-treated rats have identical enzymic properties of kynurenine 3-hydroxylase (23), which suggests that L-thyroxine acts by controlling the amount rather than the structure of kynurenine 3-hydroxylase, and analyses of time courses of changes in enzyme activities following the institution and withdrawal of thyroid hormone suggest that the decreased level of kynurenine 3-hydroxylase is due to a decreased synthesis of the enzyme protein, rather than an increased degradation (23).

Therefore, it seems that thyroid hormone may exert discriminatively its action on two different membrane systems of mitochondria.

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