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CHANGES IN CONTENT OF CYTOCHROMES c and a (a_3) IN LIVER MITOCHONDRIA OF HYPERTHYROID RATS

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Male Wistar rats aged 1, 3, 12, and 24 months were given thyroxine in a dose of 250 μ g/100 g body weight daily. After 9 days the content of cytochromes c and a (a_3) was considerably increased. An appreciable increase in the cytochrome c content was observed as early as after 24 h, whereas the content of cytochromes a (a_3) did not exceed normal even after 2 days. The content of cytochromes a (a_3) in rats aged 3, 12, and 24 months was little lower after 24 h than normally. A significant temporary increase in the ratio c/a (a_3) after 1-2 days was observed only in rats aged 12 and 24 months. An increase in the ratio c/a (a_3) with age also was demonstrated. The prospects for the use of thyroid hormones in the study of regulation of biogenesis of the mitochondria are suggested.

KEY WORDS: thyroxine; cytochromes; biogenesis of mitochondria; age.

In hyperthyroidism and thyrotoxicosis an increase is observed in the content of individual cytochromes of the respiratory chain in the liver mitochondria [3, 5, 9]. The cytochrome content is low in thyroidectomized animals [10]. Synthesis of mitochondrial cytochromes is effected by mitochondrial and cytoplasmic systems or protein synthesis, and the contribution of these two systems to the synthesis of different cytochromes differs [7]. For instance, cytochromes a and a_3 are synthesized with the participation of both systems, whereas cytochrome c is synthesized by the cytoplasmic system only. The regulatory mechanisms in both protein-synthesizing systems are sensitive to thyroid hormones [2], but the temporal characteristics of induction of synthesis of individual mitochondrial cytochromes under these circumstances have received little study. The object of this investigation was to study changes in the content of cytochromes c and a (a_3) in the liver mitochondria of rats of different ages in the early stage after administration of thyroxine.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar albino rats of four age groups: 1, 3, 12, and 24 months. The animals were given a daily intraperitoneal injection of L-thyroxine in a dose of 250 μ g/100 g body weight. The animals were killed 1, 2, and 9 days after the first injection (after 1, 2, and 9 injections respectively). Mitochondria were isolated by differential centrifugation in medium with final concentrations of: sucrose 0.3 M, Trilon B 1 mM, pH 7.2; they were washed and suspended in medium without Trilon B. The protein concentration in the mitochondrial suspensions was determined by Lowry's method in Miller's modification [13]. The ADP/O coefficient and the respiratory control in the experimental series were indistinguishable from normal during oxidation of succinate, except for a marked decrease in ADP/O in the 24-month-old rats and of the respiratory control in rats aged 3, 12, and 24 months receiving nine injections of the hormone.

The differential spectrum of the cytochromes was recorded as described in [10], with very slight changes. To a test tube containing 3.6 ml of 100 mM K-phosphate buffer, pH 7.4, were added 0.3 ml of mitochondrial

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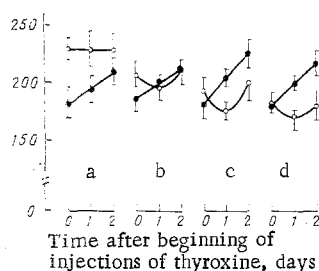


Fig. 1. Content of cytochromes in liver mitochondria of normal rats and of rats 1 and 2 days after injections of thyroxine. Ordinate, content of cytochromes (in pmoles/mg protein). Age of rats: a) 1 month, b) 3 months, c) 12 months, d) 24 months. Empty circles denote cytochrome a (a_3); filled circles cytochrome c.

TABLE 1. Ratio of Concentrations of Cytochromes c/a (a_3) in Liver Mitochondria on Rats of Different Ages under Normal Conditions and 1 and 2 Days after Administration of Thyroxine

Age, months	Ratio c/a (a_3)		
	normal	thyroxine, 1 day	thyroxine, 2 days
1	0,79±0,06 (6)	0,87±0,04 (7)	0,91±0,03 (8)
3	0,91±0,08 (7)	1,04±0,05 (8)	1,00±0,02 (8)
12	0,95±0,06 (6)	1,16±0,05 (7)	1,14±0,08 (7)
	$P < 0,1^*$	$P < 0,05$	$P < 0,1$
24	1,00±0,05 (8)	1,18±0,04	1,20±0,03 (6)
	$P < 0,05^*$	$P < 0,02$	$P < 0,01$

*Significance relative to rats aged 1 month, otherwise relative to normal.

Legend. Number of experiments in parentheses (experiments carried out in spring).

TABLE 2. Effect of Daily Administration of Thyroxine for 9 Days to Rats of Different Ages on Content of Cytochromes c and a (a_3) in Liver Mitochondria

Age, months	Cytochromes	Concentration of cytochromes, pmoles/mg protein		P
		normal	thyroxine	
1	c	218±13 (4)	282±29 (3)	<0,1
	a (a_3)	251±24 (4)	338±15 (3)	<0,05
3	c	245±15 (4)	299±12 (3)	<0,05
	a (a_3)	232±27 (4)	272±15 (3)	>0,1
12	c	230±7 (5)	311±21 (5)	<0,01
	a (a_3)	211±10 (6)	357±18 (5)	<0,001
24	c	222±16 (4)	333±18 (3)	<0,01
	a (a_3)	219±21 (4)	342±5 (3)	<0,01

Legend. Number of experiments (all experiments conducted in the fall in parentheses).

suspension (12-15 mg protein) and 0.3 ml 4% Triton X-100, and after mixing, the samples were divided equally and poured into two cuvettes. A few crystals of dithionite and 0.1 ml phosphate buffer were added to the right cuvette, 0.1 ml of 0.5 M K-ferricyanide solution to the left cuvette, and after 2-3 min the spectrum was recorded on the "Specord UV VIS" spectrophotometer. The content of cytochromes was calculated by Williams' method, as described in [1]. The detergent was used during recording of the differential spectrum not only to clarify the suspension, but also to standardize the microenvironment of the cytochromes. This is important because injection of thyroid hormones into animals causes changes in the structure of the mitochondrial membranes [8] and this, in turn, may affect the spectral characteristics of the cytochromes. The error arising with cytochrome b_5 in this connection is maximal only in respect to cytochromes b and c_1 and is very small in respect of cytochromes c and a (a_3) [6] when this method of calculation is used.

EXPERIMENTAL RESULTS

The content of cytochromes c and a (a_3) in the liver mitochondria of rats of different ages under normal conditions and 1 and 2 days after injection of thyroxine into the animals is shown in Fig. 1. Normally the content of cytochromes a (a_3) decreased with the age of the rats, whereas the content of cytochrome c was unchanged. The content of cytochromes a (a_3) in the liver mitochondria of 12- and 24-month-old rats was significantly lower than for animals aged 1 month ($P < 0.05$ and $P < 0.01$ respectively). A decrease in the content of cytochromes a (a_3) with age also was reflected in an increase in the ratio between the contents of cytochromes c/a (a_3) (Table 1).

After 1 or 2 days of injections of thyroxine into the rats an increase in the cytochrome c content was observed in the liver mitochondria (Fig. 1), and this increase was significant in the 24-month-old rats 1 day ($P < 0.05$) and in the 12- and 24-month-old rats 2 days ($P < 0.05$) after injection of thyroxine. In the rats aged 3, 12, and 24 months, some tendency toward a decrease in the content of cytochromes a (a_3) was observed 1

day after injection of thyroxine, and this was most marked in the 12-month-old animals. The difference in the direction of the changes in the content of cytochromes c and a (a_3) after 1 day (Fig. 1) also was manifested as a significant increase in the cytochrome c/a (a_3) ratio (Table 1). On the 2nd day of the experiment the content of cytochromes a (a_3) had already begun to increase, but still had not exceeded normal. In the 24-month-old rats the significant increase in the cytochrome c/a (a_3) ratio was still maintained on the 2nd day of the experiment.

The small decrease in the content of cytochromes a (a_3) 1 day after injection of thyroxine could be linked with an increase in the synthesis of other mitochondrial proteins, such as cytochrome c, α -glycerophosphate dehydrogenase [12], and an unidentified protein with molecular weight of 54,000 [4], etc., [2], and also with an increase in proteinase activity [11].

More prolonged administration of the hormone led to an increase in the content both of cytochrome c and of cytochromes a (a_3), which was most marked in the 12- and 24-month-old rats (Table 2), in general agreement with data showing an increase of 30-35% in the content of cytochrome c [5] and of 40-50% in the content of cytochrome a [9] in the liver of rats with experimental thyrotoxicosis. Under these circumstances, however, the ratio of cytochromes c/a (a_3) was no higher than normally.

With age, therefore, a decrease in the content of cytochromes a (a_3) and a corresponding increase in the c/a (a_3) ratio are observed in the liver mitochondria of rats. Prolonged administration of thyroxine to the animals caused an increase in the cytochrome content, which was greatest in rats aged 12 and 24 months, but in the early stage of development of hyperthyroidism there was a temporary increase in the c/a (a_3) ratio due to delay in the increase or even a small decrease in the content of cytochrome a (a_3), most marked in rats aged 12 months. The results point to absence of synchronization of changes in the content of the components of the mitochondrial respiratory chain in the period of transition to a new physiological state - hyperthyroidism.

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