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Effects of Thyrotoxicosis on Mitochondrial Enzymes of Rat Soleus

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Summary

Cytochrome oxidase, glycerol-3-phosphate dehydrogenase, and succinate dehydrogenase were measured in mitochondrial fractions obtained from rat soleus muscle of control and 8 week $T_3 + T_4$ treated animals. Under these conditions of prolonged treatment, there is a five-fold increase in the specific activities of both cytochrome oxidase and glycerol-3-phosphate dehydrogenase. Significant increases in total cellular mitochondrial content and enzyme activities were observed in $T_3 + T_4$ treated animals as compared to controls. These results indicate that thyrotoxicosis can induce selective changes in mitochondrial enzymes in slow twitch red (Type I) muscle fibers

Key-Words: *Thyrotoxicosis - Mitochondrial Enzymes - Rat Soleus Muscle*

Introduction

In previous studies thyroid hormone administration produces no significant effects on either hindlimb skeletal muscles or isolated gastrocnemius and vastus lateralis (Lee and Lardy 1965; van Hardeveld, Rusche, and Kassenaar 1976). Isolated soleus, however, exhibits biochemical (Winder and Holloszy

1977) and physiological (Fitts, unpublished observations) alterations which indicate that this muscle, composed predominantly of Type I fibers, is responsive to long-term thyroid hormone administration. We have re-examined the effects of thyroid hormone on rat soleus and in this communication present evidence which indicated there are significant increases in selected mitochondrial enzymes of this muscle fiber type.

Materials and Methods

Animal Care and Thyroid Administration. Female rats of a Wistar strain were obtained from Carnworth Farms and provided with food and water ad libitum and were housed in individual cages in a room maintained at 23 °C. For the administration of thyroid hormones, the rats were fed for 8 weeks a diet of powdered Purina chow with 3 mg T_4 and 1 mg T_3 /kg. Control animals which received no supplemental thyroid hormones were maintained under identical conditions.

Preparation of Homogenates. For each experiment, five rats were anesthetized with sodium pentobarbital; the soleus muscles were weighed, extracted, pooled, and homogenized in 0.1 M KCl-5mM MgCl₂-5mM EGTA-0.25 M sucrose and 25 mM imidazole, pH 6.8. Crude cell debris was re-

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moved by centrifugation for 10 min at 800 xg and the mitochondrial fraction obtained after centrifugation for 20 min at 8000 xg. The entire mitochondrial pellet was resuspended in 0.1 M KCl-0.1 M DL-glycerol-3-phosphate, pH 8.0, centrifuged for 15 min at 27,000 xg, resuspended in KCl-DL-glycerol-3-phosphate, and was used immediately for the determination of enzymatic activities.

Enzyme Assays. Cytochrome oxidase (E.C. 1.9.3.1) was measured in the spectrophotometric assay of *Phan and Mahler* (1976) and values are expressed as the first order rate constant, k, per min per milligram protein at 25 °C. Glycerol-3-phosphate dehydrogenase (E.C. 1.1.99.5) and succinate dehydrogenase (E.C. 1.3.99.1) were assayed as previously described (*Courtright* 1975) and values are expressed in terms of nanomoles product formed per minute. Protein concentrations were determined using the method of *Lowry et al.* (1951). Specific activities are expressed as units per milligram protein.

Results and Discussion

Rats maintained for 8 weeks on the T₃ and T₄ dietary regimen exhibited typical features of thyrotoxicosis, including increases in cardiac and hepatic weights and increased sarcoplasmic reticulum ATPase activities (*Winder, Baldwin, Terjung and Holloszy* 1975; and *Fitts*, unpublished observations). As seen in Table 1, the total mitochondrial protein per gram of tissue from the soleus of treated animals was significantly greater than that of control animals.

However, in contrast to the 33% increase in mitochondrial concentration, there was more than a 800% increase in cytochrome oxidase and glycerol-3-phosphate dehydrogenase activities when calculated per gram soleus. These increases apparently reflect selective induction of these mitochondrial enzymes since there is more than a five-fold increase in the specific activities (Table 1) while there is no significant change in the specific activity of succinate dehydrogenase.

The selective increase in both cytochrome oxidase and glycerol-3-phosphate dehydrogenase appears to parallel the increase in cytochrome c during thyrotoxicosis (*Winder, Baldwin, Terjung and Holloszy*

1975; *Winder and Holloszy* 1977). It is also noteworthy that both enzymes may be composed of polypeptide components synthesized on mitochondrial ribosomes (*Schatz and Mason* 1974) which are apparently synthesized at higher rates during thyrotoxicosis (*Baudry, Clot, Bouhnik, Michel and Michel* 1976).

In previous studies on the induction of glycerol-3-phosphate dehydrogenase, no significant increase in this enzyme activity could be found in extracts prepared either from skeletal muscle (*Lee and Lardy* 1965; *Lee, Tasemozi and Lardy* 1959; *Winder et al.* 1975) or from isolated gastrocnemius and vastus lateralis (*van Hardeveld, Rusche and Kassemäär* 1976). This failure to find significant changes in these muscles during thyrotoxicosis may be due to the fact that these samples were primarily composed of Type IIA and IIB (fast twitch fiber types) which differ in a number of biochemical and physiological parameters from the slow twitch muscle (Type I), which composes 85% of rat soleus (*Brooke and Kaiser* 1970; *Peter, Barnard, Edgerton, Gillespie and Stemple* 1972; *Ariano, Armstrong and Edgerton* 1973). It seems likely that the response of soleus to prolonged T₃ + T₄ administration provides yet another biochemical characterization of this fiber type and may indicate that soleus has different receptors for these hormones.

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Table 1 Effect of thyroid hormone on the mitochondrial and enzyme content of soleus

	Control	Experimental
Mitochondria (mg/gm)	17.1 \pm 0.6	25.7 \pm 3.5*
Cytochrome oxidase (k/mg protein)	21.5 \pm 5.5	112 \pm 12**
Glycerol-3-phosphate dehydrogenase (nmol/min/mg protein)	4.8 \pm 1.4	28.3 \pm 4.1***
Succinate dehydrogenase (nmol/min/mg protein)	55 \pm 16	44 \pm 6

Values are means \pm SE.

Results are expressed per gram wet weight of pooled soleus muscles from 5 animals or per milligram mitochondrial protein, N = 4.

*Control vs. T₃ + T₄-treated, P < 0.1

**Control vs. T₃ + T₄-treated, P < 0.002

***Control vs. T₃ + T₄-treated, P < 0.001

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