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THYROXINE-INDUCED CHANGES IN RAT LIVER MITOCHONDRIAL UBIQUINONE

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Ubiquinone was extracted from liver mitochondria isolated from euthyroid and hyperthyroid rats. The redox state of ubiquinone was determined during States III and IV respiration with succinate or glutamate-malate substrates. Ubiquinone was more reduced during State III or IV in the hyperthyroid mitochondria with either substrate. Furthermore, the concentration of ubiquinone increased in the hyperthyroid rats.

A major effect of thyroid hormones is to increase mitochondrial respiration. This increased respiration is due to many complex changes in the number and activity of mitochondrial respiratory components. These changes include increases in the concentration of electron transport components such as ubiquinone (1), some of the cytochromes (2-4) as well as associated enzymes such as the atractyloside-sensitive adenine nucleotide carrier (5). Information of this sort led to the proposal that the components of the respiration chain respond as a respiratory unit to the animals' thyroid status (3). However, recently we (4) and others (6) have demonstrated that the concentration of at least one component, cytochrome $\underline{c}_1$, does not increase in the hyperthyroid state. Thyroid-enhanced respiration is also associated with changes in the activity of the components of the electron transport chain. In hypothyroid rats treated with thyroid hormones, the initial increase in respiration occurs without increased cytochrome content but with more reduced cytochromes (7). Furthermore, we demonstrated in hyperthyroid rats, that all of the cytochromes were more reduced during State III respiration and all, except $\underline{b}$, were more

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reduced during State IV respiration (4). Thus, thyroid hormone induced changes in mitochondrial respiration are due to alterations in both the concentration and the activity of the electron transport components.

In this paper, we have investigated the effects of thyroxine treatment upon the content and redox state of ubiquinone, the coenzyme of the bc_1 complex. We confirm the reported (1) increase in content of ubiquinone during hyperthyroidism. Furthermore, we demonstrated a thyroxine-induced altered redox state for ubiquinone during States III and IV respiration.

MATERIAL AND METHODS

Treatment of Rats. Male Sprague Dawley rats (Sasco Inc., Omaha, NE) initially weighing 175-250 g were used throughout this study. The animals were given laboratory chow and water ad libitum. Rats were made hyperthyroid by daily subcutaneous injections, for 10 days, of 15 µg thyroxine/100 g body weight. The thyroxine was dissolved in 50 mM NaOH at a concentration of 150 µg thyroxine/ml of NaOH. Control rats were injected daily for 10 days with 50 mM NaOH. Twenty-four hours after the last injection, the rats were killed by decapitation. Blood was collected from the neck to determine serum thyroxine levels. The thyroid status was checked by a Thyroxine Radioimmunoassay kit (Ventrex Laboratories, Portland, ME).

Isolation and Treatment of Mitochondria. Liver mitochondria were prepared according to the procedure of Johnson and Lardy (8). Oxygen consumption was measured polarographically at 37° C as described previously (9) in a Gilson Oxygraph 5/6 (Gilson Medical Instruments, Middleton, WI) with succinate (6 mM) or glutamate-malate (6 mM each) as substrates.

Isolation and Determination of Ubiquinone. Mitochondrial ubiquinone was isolated by the method of Kroger and Klingenberg (10). Mitochondria were extracted during State III or State IV respiration. Ubiquinone was extracted into 60% (v/v) methanol and light petroleum. This method extracts ubiquinone with the redox state corresponding to the metabolic state of the preparation. State III was measured during active oxidative phosphorylation in the presence of substrate and 0.5 mM ADP. State IV was measured in the presence of substrate but in the absence of ADP. The redox state of the ubiquinone was calculated by comparing the percent oxidized during respiration to the complete oxidation resulting from freeze-thawing a sample of the mitochondria. Total reduction of the samples was caused by the addition of potassium borohydride and followed at 280-289 nm on an Aminco DW-2a spectrophotometer (American Instrument Co., Silver Springs, MD). A difference extinction coefficient of 8.8 $mM^{-1}.cm^{-1}$ was used to calculate ubiquinone cocentration (11).

Other Methods. Protein was measured by the Biuret method (12) using bovine serum albumin as the standard. All chemicals were reagent grade and obtained from Sigma Chemical Co. (St. Louis, MO) except methanol and petroleum ether which were spectrophotometric grade obtained from Aldrich Chemical Co. (Milwaukee, WI). Statistical differences were determined by the Student's t test. Results were represented as the mean ± standard error.

RESULTS

Thyroxine treatment resulted in a hyperthyroid state demonstrated by changes in thyroxine serum levels and by changes in mitochondrial respiration. The thyroxine serum concentrations were 7.5 ± 0.4 µg thyroxine/dl for ten

control rats and 26.1 ± 2.2 μg thyroxine/dl for ten hyperthyroid rats ($p < 0.001$). Mitochondrial respiration was also determined using succinate or glutamate-malate as substrates. Succinate respiration during State III was 226.9 ± 13.1 vs 331.0 ± 21.5 nmoles O_2/minute/mg protein for ten euthyroid and ten hyperthyroid rats, respectively ($p < 0.005$). Succinate respiration during State IV was 51.1 ± 5.3 vs 110 ± 13.8 nmoles O_2/minute/mg protein for the euthyroid and hyperthyroid rats, respectively ($p < 0.005$). The resulting respiratory control ratios (State III/State IV) were 4.4 ± 0.2 for the euthyroids and 3.0 ± 0.2 for the hyperthyroids ($p < 0.005$). Glutamate-malate respiration during State III respiration was 92.4 ± 13.7 vs 138 ± 13.7 nmoles O_2/minute/mg protein ($p < 0.005$), and during State IV respiration was 20.7 ± 2.3 vs 35.8 ± 2.4 nmoles O_2/minute/mg protein ($p < 0.005$) for the euthyroid and hyperthyroid rats, respectively. The resulting respiratory control ratios were 4.5 ± 0.2 for the euthyroids and 3.9 ± 0.2 for the hyperthyroids ($p < 0.05$).

The content of ubiquinone was increased by 75% in the mitochondria from thyroxine-treated rats as shown in Table 1. Not only was the content of

TABLE 1. THYROXINE-INDUCED CHANGES IN MITOCHONDRIAL UBIQUINONE

Ubiquinone Content	
Treatment	(nmoles ubiquinone/mg protein)
Control (n = 10)	0.62 ± 0.02
+ Thyroxine (n = 10)	1.08 ± 0.06*

Ubiquinone Percent Reduction		
Treatment	State III	State IV
Succinate Substrate		
Control (n = 10)	30.6 ± 3.4%	62.0 ± 2.9%
+ Thyroxine (n = 10)	45.9 ± 2.9%*	71.7 ± 1.8%*
Glutamate-Malate Substrate		
Control (n = 10)	9.3 ± 2.2%	22.3 ± 3.5%
+ Thyroxine (n = 10)	27.6 ± 3.5%*	39.7 ± 5.8%*

*Significantly different compared to controls ($p < 0.001$).

n = Number of animals per group.

ubiquinone increased by thyroxine treatment, but the redox state of ubiquinone during respiration was also altered. Table 1 shows that ubiquinone was more reduced in the hyperthyroid mitochondria respiring either succinate or glutamate-malate. Furthermore, ubiquinone was more reduced during both State III and State IV respiration.

DISCUSSION

Thyroid hormones affect mitochondrial respiration by altering the concentrations of some of the components of the chain (1-5) as well as by altering the redox state of the components (4,7). We recently demonstrated that thyroxine treatment resulted in increased concentrations of cytochromes b, c, and a.a_3 but not c_1, and thyroxine also altered the redox state of the cytochromes (4). During State III respiration with succinate, cytochromes b, c, and c_1 were more reduced in the hyperthyroid rats. While in State IV respiration, cytochromes c and c_1 were more reduced and cytochrome b oxidized (4). The lack of change in the content of c_1 and the altered redox state of b suggests the bc_1 complex may be a major site of regulation of respiration by thyroid hormones.

To investigate additional aspects of thyroid control of respiration, we examined the effects of hyperthyroidism on ubiquinone. Mitochondrial ubiquinone serves as the coenzyme for the bc_1 complex and is an essential component of the electron transport chain which functions as both an electron carrier and a proton carrier (13). Its location in the chain combines the paths of electron flow from NADH and succinate.

Early studies showed that thyroxine treatment increased the content of ubiquinone (1) and our data (Table 1) confirms this observation. Furthermore, we found a more reduced redox state for the coenzyme which was seen during both State III and State IV respiration with either succinate or glutamate-malate as substrates. This could be due to an increased rate of ubiquinone reduction resulting from enhanced electron flow from the sites of substrate oxidation. Such an increased electron flow has been suggested during hyperthyroidism for both succinate (4) and NAD-linked substrates (3).

The more reduced redox state of ubiquinone found during hyperthyroidism must be reconciled to the altered redox states of the cytochromes as well as to the increased rates of respiration. Previously we proposed that during State III respiration in hyperthyroids, there was an equilibrium existing between the rates of substrate oxidation and the redox states of the cytochromes (4). The more reduced redox state of ubiquinone in the hyperthyroids also appears to be in equilibrium with the other components of the electron transport chain during State III respiration.

During State IV respiration, the electron transport chain appears to be in disequilibrium in thyroxine-treated animals, demonstrated by relative oxidation of cytochrome b. The apparent cause of this disequilibrium may be "loose coupling" near cytochrome b as suggested by Hassinen et al (14). The present study suggests that ubiquinone is in equilibrium with cytochrome c_1 but not with cytochrome b. Thus, the site of the loose coupling appears to involve ubiquinone and cytochromes b and c_1.

There are several slightly varying forms of the ubiquinone cycle which explain electron and proton flow through the bc_1 complex (15). The disequilibrium during State IV in the redox states of the proton and electron carrier, ubiquinone, and the electron carrier, cytochrome b, suggests proton and electron movement through ubiquinone may be uncoupled from electron movement through cytochrome b. Enhanced substrate oxidation with this "uncoupling" could result in relative oxidation of cytochrome b, enhanced reduction of ubiquinone and cytochrome c_1, and increased rate of H^+ efflux. The result of an increased rate of H^+ efflux would be to increase the protonic electrochemical potential difference across the mitochondrial respiratory membrane. Such an increase in the protonmotive force has been observed in thyroxine treated rats (16,17). This increase, coupled to a thyroid hormone induced increased passive permeability of the mitochondrial inner membrane to protons (18) could account for the relatively greater stimulation of State IV respiration in thyroxine-treated rats as reflected by the decreased respiratory control ratio (4).

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