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EFFECTS OF THYROID HORMONES ON THE BYPASSES OF THE ANTIMYCIN A BLOCK IN THE bc_1 COMPLEX OF RAT LIVER MITOCHONDRIA

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The effect of thyroid hormones on the electron flow through the bc_1 complex of rat liver mitochondria was studied using two dye bypasses of the Antimycin A block of the bc_1 complex by the method of Alexandre and Lehninger (Biochim. Biophys. Acta 767:120; 1984). Bypass respiration rates with both DCIP (2,6-dichlorophenolindophenol) and TMPD (N,N,N',N'-tetramethyl-*p*-phenylenediamine dihydrochloride) were elevated in the hyperthyroid rats and depressed in the hypothyroid groups compared to the euthyroid controls. T_3 treatment of hypothyroid rats returned the bypass rates to control levels in 24 hours with the TMPD dye but not for the DCIP. This further demonstrates that different portions of the bc_1 complex respond individually to the thyroid state.

The bc_1 complex of the mitochondrial electron transport system appears to be thyroid hormone sensitive. The thyroid state affects both the content and the redox state of the cytochromes and ubiquinone of the bc_1 complex (1-11). Electron flow through the bc_1 complex remains under study. Wikstrom and Berden showed that electron flow in the bc_1 complex could not occur by a linear scheme (12). Mitchell proposed a divergent pathway for electron flow, the Q cycle in the bc_1 complex (13,14). Dyes have been used

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Abbreviations: DCIP: (2,6-dichlorophenolindophenol) and TMPD: (N,N,N',N'-tetramethyl-*p*-phenylenediamine dihydrochloride).

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to examine electron flow in this complex (12,15). Alexandre and Lehninger (15) used the dyes, DCIP and TMPD. In this paper, we couple the methods of Alexandre and Lehninger (15) to the effects of thyroid hormone treatment in order to examine the effects of thyroid hormone on electron flow through the $\underline{bc}_1$ complex.

MATERIALS AND METHODS

Male Sprague-Dawley rats initially weighing 175-250g were used throughout the studies. The animals were given laboratory chow and water ad libitum. Rats were made hyperthyroid by daily subcutaneous injections, for ten days, of L-thyroxine (T_4) at 15μg/100g body weight. The T_4 was dissolved in 10mM NaOH at a concentration of 150μg/ml. Control rats were injected for ten days with the vehicle only. Rats were made hypothyroid by the addition of PTU (6-n-propylthiouracil), 0.05% (w/v) to their drinking water for 15-20 days. For 3,3',5-triiodo-L-thyronine (T_3) treatment of rats, a single dose of T_3, 40μg/100g body weight was injected subcutaneously. Liver mitochondria were isolated by the method of Johnson and Lardy (16). Respiratory rates were defined according to Chance and Williams (17). Serum T_3 and T_4 levels were determined by radioimmunoassay. Analysis of variance (ANOVA), was performed on data from pooled groups of rats.

Studies of the bypass of the antimycin block by DCIP (2,6-dichlorophenolindophenol) or TMPD (N,N,N',N'-tetramethyl-*p*-phenylenediamine dihydrochloride) were performed according to the methods of Alexandre and Lehninger (15). Solutions of both dyes were made daily.

RESULTS

Table 1 shows the effects that thyroid hormone treatments had upon serum hormone levels and mitochondrial respiration. The serum hormone levels were consistent with the treatment regimen. Hyperthyroid (T_4 treated) rats had significantly higher serum T_3 and T_4 levels compared to the euthyroids. Hypothyroid rats showed hormone values which were below the assay's sensitivity. In the T_3-treated hypothyroid rats, the serum T_4 level was also below the assay's sensitivity while the serum T_3 was markedly elevated. These changes are reflected in the changes of mitochondrial respiration. Hyperthyroid rates were significantly elevated while the hypothyroid rates were decreased. The T_3-treated hypothyroid rats gave respiratory results which were not significantly different from the euthyroid rates. There were no significant differences in the P:O ratios between the four groups (data not shown). These data show that the treatment of the rats altered their thyroid hormonal concentrations and their mitochondrial metabolism.

Table 2 shows the results of the DCIP and the TMPD bypasses of the antimycin A block in whole mitochondria isolated from the various thyroid hormone groups. The bypass respiration of mitochondria from hyperthyroid rats was significantly higher compared to the euthyroid controls. This was true for both the DCIP and the TMPD dyes with either succinate or glutamate-malate as substrates. The respiratory rates of mitochondria from the hypothyroid group was significantly lower than the euthyroids for

Table I. The effects of various thyroid treatments upon serum hormone levels and mitochondrial respiratory parameters with succinate as the substrate

ASSAY	EUTHYROID	HYPER-THYROID	HYPO-THYROID	HYPO-THYROID+T_3
SERUM T_3 ng/ml	0.7+/-0.2	2.1+/-0.1**	<0.09^{**,bb}	10.0+/-0.6^{**,aa,bb}
SERUM T_4 ug/dl	8.9+/-0.9	16.2+/-0.9**	<0.5^{**,bb}	<0.5^{**,bb}
STATE III †	233+/-11	278+/-12*	186+/-19^{*,bb}	239+/-11^{a}
STATE IV †	63+/-6	86+/-5**	46+/-5^{*,bb}	62+/-5^{a,bb}
RESPIRATORY CONTROL RATIO	3.9	3.2	4.0	3.9
n =	7	5	6	7

n = Number of animals tested.
† = nmoles O_2/minute/mg protein
* = $p < 0.05$ Significantly different from euthyroids.
** = $p < 0.01$ Significantly different from euthyroids.
a = $p < 0.05$ Significantly different from hypothyroids.
aa = $p < 0.01$ Significantly different from hypothyroids.
b = $p < 0.05$ Significantly different from hyperthyroids.
bb = $p < 0.01$ Significantly different from hyperthyroids.

both the DCIP and the TMPD bypasses. There was a significant difference between the euthyroid group and the T_3-treated hypothyroid group for the DCIP bypass, but not the TMPD bypass, with both electron sources. The bypass rates of the T_3-treated hypothyroid group were significantly higher than the hypothyroid rates using TMPD, but not DCIP. Similar results and rates were observed when the mitochondria were sonicated prior to the dye bypass assays indicating that the intact mitochondrial membranes did not exclude the dyes form the sites of action (data not shown).

DISCUSSION

In this paper we have examined the effects of the thyroid status on mitochondrial respiration resulting from the use of two different artificial electron carriers to bypass the Antimycin A electron block in the $\underline{bc}_1$ complex. This approach was introduced by Wikstrom and Berden (12). Alexandre and Lehninger (15), showed that the block was bypassed by DCIP and TMPD through two separate routes within the Q cycle. The

Table 2. Effects of the thyroid status on the respiratory rates during the DCIP and the TMPD bypass of the antimycin A block in whole mitochondria using either succinate or glutamate/malate as the substrates

Thyroid status	DCIP Succinate †	DCIP Glutamate/ Malate †	TMPD Succinate †	TMPD Glutamate/ Malate †
Euthyroid n=7	297.9± 28.6	205.9± 21.9	233.3± 26.2	58.0± 9.5
Hyper-thyroid n=5	396.2 21.0**	322.3± 7.6**	403.3± 32.6**	112.0± 21.1**
Hypo-thyroid n=6	169.0± 11.6**,bb	131.1± 5.7**,bb	112.3± 13.2**,bb	43.2± 4.4bb
Hypo-thyroid + T_3 n=7	208.3± 15.9**,bb	151.7± 8.3*,bb	243.9± 20.8aa,bb	65.5± 5.7a,bb

† = nmoles O_2 / minute / mg protein
* = $p < 0.05$ Significantly different from euthyroids.
** = $p < 0.01$ Significantly different from euthyroids.
a = $p < 0.05$ Significantly different from hypothyroids.
aa = $p < 0.01$ Significantly different from hypothyroids.
b = $p < 0.05$ Significantly different from hyperthyroids.
bb = $p < 0.01$ Significantly different from hyperthyroids.
n = Number of animals tested.

antimycin-inhibited electron flow from succinate to oxygen could be reactivated by an artificial electron donor (reduced DCIP) or by an artificial electron acceptor (oxidized TMPD) (15). Reduced DCIP appears to activate electron transfer from succinate to cytochrome c in the presence of antimycin by reducing the ubisemiquinone back to ubiquinol in the early steps at center P. Oxidized TMPD (WB^+, Wurster's blue) acts as an oxidant for reduced species that accumulate in the presence of antimycin, i.e., either ubisemiquinone or the reduced cytochromes b (15).

Thyroid hormone status alters the structure and function of the bc_1 complex. Hypothyroid rats have decreased levels of cytochromes b and c which increase following thyroid hormone replacement (1,2). An initial increase in respiration seen in hypothyroid rats treated with thyroid hormones occurs in the absence of an increased cytochrome content but in the presence of an increased rate of cytochrome reduction (9,10). This

increase in respiration may involve the activation of the $\underline{bc}_1$ complex (10,11). Hyperthyroid rats have elevated concentrations of ubiquinone (3,4) and cytochromes $\underline{b}$ and $\underline{c}$ (2,5-7), but not cytochrome c_1 (5,8). During State III respiration, hyperthyroidism results in enhanced reduction of ubiquinone and the cytochromes. During State IV respiration in hyperthyroid mitochondria, cytochrome $\underline{b}$ is more oxidized, while cytochromes $\underline{c}$ and $\underline{c}_1$ and ubiquinone are more reduced (4,5). These data indicate that the $\underline{bc}_1$ complex is involved in the regulation of respiration by thyroid hormones.

Mutvei and Nelson (10) showed that the increases in the levels of $\underline{bc}_1$ subunits in hypothyroid rats required 3-6 days of high T_3 doses. In contrast, the respiration increased under conditions in which no accumulation of the subunits was observed. They suggest that the transition from the hypothyroid state to the euthyroid state involves two parts: an early activation of respiration controlled either directly by the hormone or via hormone-responsive signal and a later increase in respiration which requires stimulation of mitochondrial protein synthesis and the accumulation of mitochondrial complexes.

Our T_3-treated hypothyroid DCIP bypass results suggest thyroid sensitive changes in the $\underline{bc}_1$ complex. The changes do not appear due to either a lack of dye or to a lack of the dye's electron carrying capacity since the hyperthyroid group for both dyes showed increased rates of bypass respiration. The changes do not appear due to an altered permeability to the dye by the mitochondria since equivalent results were seen in sonicated mitochondria (data not shown). This lack of increase in the DCIP bypass rate in T_3-treated hypothyroid is not due to decreased substrate dehydrogenase activity since mitochondrial respiration increased in the presence of TMPD. Rather, the differences between the TMPD bypass and the DCIP bypass appears due to thyroid hormone specific changes in electron flow through a portion of the $\underline{bc}_1$ complex.

One explanation for the difference between the two bypass rates in the T_3-treated hypothyroid state is as follows. The 24 hour increase in respiration seen in the hypothyroid rats treated with T_3 appears partly due to activation of the components either directly, or by an alteration in their environment (10,11). DCIP may be interacting with this process. The interaction site would occur where the dye acts in the complex, the center P (15). Subunit VII of the complex, a ubiquinone-binding protein, is actively involved in electron transfer at center P of the $\underline{bc}_1$ complex (18). The dye could be interfering with an activation of the ubiquinone-binding protein activity or with electron transfer involving an activated binding protein and bound ubisemiquinone. The TMPD results suggest that TMPD does not interfere in the activation of the $\underline{bc}_1$ complex and that WB^+ may accept the electrons from the reduced cytochromes $\underline{b}$ rather than from the ubisemiquinone. The current studies are unable to establish how DCIP interferes with electron transport at

center P of the $\underline{bc}_1$ complex in the T_3-treated hypothyroid mitochondria. Additional studies are needed to determine the role of thyroid hormones in the control of mitochondrial respiration.

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