Is respiratory activity in the brain mitochondria responsive to thyroid hormone action?: a critical re-evaluation

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The effects of *in vivo* treatment with graded doses (0.5–1.5 μg/g body weight) of thyroid hormones, tri-iodothyronine (T_3) and thyroxine (T_4), for 4 consecutive days to euthyroid rats on the respiratory activity of isolated brain mitochondria were examined. T_4 stimulated coupled State-3 respiration with glutamate, pyruvate + malate, ascorbate + tetramethyl-*p*-phenylenediamine and succinate, in a dose-dependent manner; T_3 was effective only at the highest (1.5 μg) dose employed. T_4 was more effective than T_3 in stimulating respiratory activity. State-4 respiratory rates were in general not influenced except in the case of the ascorbate + tetramethyl-*p*-phenylenediamine system. Primary dehydrogenase activities, i.e. glutamate dehydrogenase, malate dehydrogenase and succinate dehydrogenase, were stimulated about 2-fold; interestingly mitochondrial but not cytosolic malate dehydrogenase activity was influenced under these conditions. The hormone treatments did not greatly influence the mitochondrial cytochrome content. The results therefore suggest that thyroid hormone treatment not only stimulates primary dehydrogenase activities but may also directly influence the process of mitochondrial electron transfer.

INTRODUCTION

Although the role of thyroid hormones in brain development and the function of the central nervous system is generally well recognized [1–3], reports on the effects of thyroid hormones on respiration are often contradictory [2,4–8]. Thus it has been reported that, after thyroid hormone treatment, O_2 consumption in liver, heart and diaphgram increased significantly, whereas that in brain, spleen and testes did not [4]. It has also been reported that hypothyroidism did not significantly affect brain respiration with glucose as substrate [5]. On the basis of such observations, brain has generally been considered to be unresponsive to thyroid hormone action. In contrast, Reiss et al. [6] reported that respiration in the neonatal, but not adult, rat brain was stimulated by thyroid hormone treatment; Schwartz and Oppenheimer [7], were, however, unable to confirm these findings. In addition, it has been repeatedly emphasized that, in brain unlike in responsive tissues, α-glycerophosphate dehydrogenase and malate dehydrogenase fail to respond to thyroid hormone administration [9].

We previously reported that thyroid deficiency adversely affects respiration in rat brain mitochondria [10] and that treatment with thyroid hormones produces a restorative effect [11]. Moreover, in both euthyroid and hypothyroid neonatal and 11-day-old rats, we demonstrated a marked stimulation of the respiratory activity by tri-iodothyronine (T_3) treatment [8]. However, thyroid hormone effects on mitochondrial respiratory activity in euthyroid adult animals have not been examined thus far. Because the number of nuclear T_3 receptors in the brain is comparable with that in responsive tissues such as liver and kidney [9], it is clearly important to discover whether respiration in the brain does, indeed, not respond to thyroid hormones. We have therefore studied the effects of treatment of euthyroid rats with graded doses of T_3 and L-thyroxine (T_4) on respiratory activity of isolated brain mitochondria employing a variety of respiratory substrates. The present paper reports the results of these studies.

MATERIALS AND METHODS

Chemicals

T_3, T_4, ADP, sodium salts of L-glutamic acid, pyruvic acid, L-malic acid, L-ascorbic acid and deoxycholic acid, rotenone and Triton X-100 were purchased from Sigma Chemical Co. *NNN′N′*-Tetramethyl-*p*-phenylenediamine (TMPD) was from BDH. All other reagents were of the highest purity commercially available.

Treatment with thyroid hormones

Male albino Wistar rats weighing between 200 and 210 g were injected with 0.5, 1.0 or 1.5 μg of T_3 or T_4/g body weight for 4 consecutive days between 08:30 and 09:30 h [12,13]. Hormone solutions were prepared fresh daily in 0.9% NaCl containing 5 mM NaOH [12], and concentrations were adjusted such that 100 μl was injected/100 g body weight. Controls received comparable volumes of the vehicle. Animals had free access to food and water. A daily record of body weights was maintained. Animals were killed on the fifth day (between 08:30 and 09:30 h) and brain mitochondria were isolated.

Isolation of mitochondria and measurement of oxidative phosphorylation

Brain mitochondria were isolated as described previously using 0.3 M mannitol containing 1 mM EDTA, pH 7.4, as the isolation medium [13,14]. Centrifugations were carried out in a Sorvall RC-5C centrifuge at 0–4 °C. Mitochondrial pellets were suspended in the isolation medium to yield a protein concentration of 10–12 mg/ml.

Oxidative phosphorylation was measured at 25 °C using a Clark electrode as described previously [14]. The reaction medium (1.3 ml) contained 0.3 M mannitol, 20 mM KCl, 0.2 mM EDTA, 5.0 mM potassium phosphate buffer and 10 mM Tris/HCl, all at

Abbreviations used: T_3, 3,3′,5-tri-iodo-L-thyronine; T_4, L-thyroxine; TMPD, *NNN′N′*-tetramethyl-*p*-phenylenediamine.

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Table 1 Effects of thyroid hormones on oxidative phosphorylation in rat brain mitochondria using glutamate, pyruvate plus malate, succinate, and ascorbate plus TMPD as the electron-donor systems

T_3 and T_4 were given for 4 consecutive days. Other experimental details are as given in the text. Results are means ± S.E.M. of 16–20 observations for the individual data points. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$, compared with the control.

		Respiration rate (nmol of O_2/min per mg of protein)							
	Dose (μg/g	Glutamate		Pyruvate + malate		Succinate		Ascorbate + TMPD	
Treatment	body weight)	+ ADP	− ADP	+ ADP	− ADP	+ ADP	− ADP	+ ADP	− ADP
Control	–	31.0 ± 1.1	3.8 ± 1.1	46.1 ± 2.7	5.8 ± 0.5	60.1 ± 2.0	23.2 ± 2.5	71.7 ± 4.5	41.0 ± 2.1
T_3	0.5	33.2 ± 1.9	3.8 ± 0.4	46.9 ± 1.9	5.3 ± 0.6	64.5 ± 3.1	29.1 ± 2.9	71.4 ± 3.2	38.8 ± 2.3
T_3	1.0	28.8 ± 1.2	3.0 ± 0.4	42.8 ± 1.4	6.9 ± 0.6	57.6 ± 1.6	24.8 ± 1.8	56.1 ± 2.0**	30.4 ± 2.1***
T_3	1.5	36.3 ± 2.2*	2.9 ± 0.5	64.5 ± 5.9***	5.9 ± 0.6	84.7 ± 3.8***	32.4 ± 4.1	105.4 ± 4.5***	61.4 ± 2.7***
T_4	0.5	28.0 ± 1.2	3.6 ± 0.6	48.6 ± 2.3	7.8 ± 1.1	69.0 ± 3.5*	23.7 ± 3.2	121.6 ± 4.6***	65.7 ± 3.8***
T_4	1.0	31.8 ± 1.7	2.9 ± 0.4	56.3 ± 2.3**	6.5 ± 1.1	79.6 ± 3.0***	32.1 ± 4.0	120.2 ± 5.7***	66.8 ± 3.8***
T_4	1.5	53.6 ± 3.0***	3.1 ± 0.4	75.1 ± 3.3***	5.3 ± 0.5	82.7 ± 4.2***	30.8 ± 4.0	99.6 ± 3.6***	59.8 ± 2.5***

pH 7.4. Saturating concentrations of substrates, i.e. glutamate (10 mM), pyruvate (10 mM) plus malate (1 mM), succinate (10 mM) and ascorbate (10 mM) plus TMPD (0.1 mM), were used. State-3 respiratory rates (initiated by addition of 120 nmol of ADP in 15–20 μl) and State-4 respiratory rates (after the depletion of added ADP) were recorded. ADP/O ratios were calculated from the amount of ADP added and the corresponding O_2 consumption as described previously [10].

Other methods

Cytochrome contents were estimated from the difference spectra of Triton X-100/deoxycholate-solubilized mitochondria as described previously [14]. Glutamate dehydrogenase, malate dehydrogenase and succinate dehydrogenase activities were measured spectrophotometrically [15]. Protein was estimated by the method of Lowry et al. [16] with BSA as standard. Serum T_3 and T_4 levels were measured by radioimmunoassay [13,14]. Statistical evaluation of the data was by Student's *t* test.

RESULTS

During the 4-day treatment period, control animals gained an average of 33 g in body weight (initial body weight 202 ± 4.4 g; final body weight 237.9 ± 4.5 g; means ± S.E.M.); this was prevented by injection of 0.5 or 1.0 μg of T_3, and 1.5 μg of T_3 caused a net weight loss (10 g). The effects of T_4 were less marked, with gains of 21, 14 and 7 g on treatment with 0.5, 1.0 and 1.5 μg doses respectively. Thus the effects of T_3 and T_4 were distinguishable even with respect to a gross parameter such as body weight, the difference emphasizing the degree to which T_3 generally exerts greater catabolic effects than does T_4 [17].

Effects of T_3 on oxidative phosphorylation

Table 1 summarizes the effects of T_3 and T_4 on oxidative phosphorylation in rat brain mitochondria. With glutamate as the respiratory substrate, State-3 respiration was stimulated by a maximum of 17% and only at the highest dose of T_3 employed (1.5 μg). State-4 respiration was unaffected at all concentrations of T_3. The ADP/O ratio for the control was 2.98 ± 0.07; in the experimental group the values decreased somewhat but were still in the expected theoretical range (results not given).

A similar situation obtained when pyruvate plus malate was employed as the electron-donor system; treatment with 1.5 μg of T_3 led to a substantial increase (+40%) in State-3 respiration. Likewise, when succinate was the electron donor, only the 1.5 μg dose produced a similar increase (+41%) in State-3 respiratory rate. T_3 treatment did not influence State-4 respiratory rates for either of these substrates.

For ascorbate + TMPD the highest dose of T_3 produced a 47% increase in State-3 respiration; however, the 1.0 μg dose caused a transient decrease (−22%).

Even with pyruvate plus malate, succinate, and ascorbate + TMPD as the electron-donor systems, the ADP/O ratios were in the expected theoretical ranges in the experimental groups, although at times somewhat lower than the control (results not given).

Effects of T_4 on oxidative phosphorylation

Table 1 shows that with glutamate as the substrate, T_4 treatment produced an effect similar to that with T_3, but with a greater stimulation (+73%) at the highest dose of the hormone (1.5 μg of T_4). With pyruvate plus malate as the electron-donor system, stimulation (+22%) was noted at 1.0 μg, with an additional increase (+63%) at 1.5 μg. In the case of succinate, increased State-3 respiration could be seen at the lowest dose (0.5 μg) with progressively greater increases at higher concentrations. Finally, when ascorbate + TMPD was used as the electron-donor system, T_4 exerted a maximum stimulatory effect on State-3 respiration (+70%) at the lower two concentrations, whereas at 1.5 μg the effect was somewhat diminished.

It is thus apparent from the data in Table 1 that T_4 was generally more effective than T_3 in stimulating State-3 respiration, producing its effect at lower doses and giving rise to a larger final rate increase at higher doses. It is also clear that the State-4 rates of respiration were generally unaffected except in the case of the ascorbate + TMPD system.

For all the substrates, the ADP/O ratios in the experimental group were generally not affected and compared well with those for controls (results not given).

Mitochondrial cytochrome contents

The content of aa_3 cytochromes decreased in brain mitochondria (−15%) from animals receiving 1.0 μg of T_3 (Table 2). It is possible that this decrease may account for the diminished State-3 and -4 respiratory rates noted above for ascorbate + TMPD

Table 2 Effect of thyroid hormone treatment on cytochrome content of rat brain mitochondria

Experimental details are given in the text and Table 1. Results are means ± S.E.M. of the number of observations indicated in the parentheses. *$P < 0.05$, **$P < 0.02$ and ***$P < 0.001$, compared with the control.

Treatment	Dose (μg/g body weight)	Cytochrome content (pmol/mg of protein)		
		aa_3	b	$c+c_1$
Control (14)	–	152.8 ± 6.8	115.3 ± 6.9	341.0 ± 13.4
T_3 (19)	0.5	158.6 ± 6.1	138.5 ± 11.5	381.2 ± 12.7*
T_3 (22)	1.0	129.6 ± 7.1*	123.7 ± 4.1	296.7 ± 8.5***
T_3 (14)	1.5	150.9 ± 7.9	100.7 ± 10.2	295.8 ± 11.4**
T_4 (12)	0.5	152.0 ± 7.4	144.1 ± 8.9**	359.8 ± 13.2
T_4 (10)	1.0	147.5 ± 17.6	102.4 ± 6.9	370.4 ± 13.1
T_4 (22)	1.5	150.3 ± 11.1	95.9 ± 5.4*	358.3 ± 14.6

Table 4 Serum T_3 and T_4 levels in rats after thyroid hormone treatment

Experimental details are given in the text and Table 1. Results are means ± S.E.M. of four independent observations in each group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.002$ and ****$P < 0.001$, compared with the control.

Treatment	Dose (μg/g body weight)	T_4 (μg/dl)	T_3 (ng/dl)
Control	–	6.10 ± 0.53	124.5 ± 1.71
T_3	0.5	3.63 ± 0.29**	132.5 ± 1.44**
T_3	1.0	2.83 ± 0.10****	1500.0 ± 40.80****
T_3	1.5	3.13 ± 0.23***	2850.0 ± 86.60****
T_4	0.5	8.50 ± 0.82*	740.0 ± 11.55****
T_4	1.0	16.63 ± 0.59****	1050.0 ± 35.35****
T_4	1.5	29.50 ± 2.02****	1900.0 ± 91.29****

(Table 1). The cytochrome *b* content increased by 25% in the group receiving 0.5 μg of T_4, but displayed a steady decrease as the dose increased. Similarly, cytochrome $c + c_1$ content increased in animals receiving 0.5 μg T_3, but then decreased at higher doses of the hormone.

Dehydrogenase activities

The activities of various dehydrogenases are summarized in Table 3. Mitochondrial glutamate dehydrogenase, malate dehydrogenase and succinate dehydrogenase activities were approximately double after hormone treatment. Stimulation of glutamate dehydrogenase and malate dehydrogenase was somewhat diminished at higher doses of T_4, but the activities were still more than sufficient to sustain the higher rates of respiration noted above for these substrates (see Table 1). Interestingly, cytosolic malate dehydrogenase was unaffected by thyroid hormone treatment.

Thyroid hormone levels in serum

In concomitant studies, we measured the levels of T_3 and T_4 in serum using the radioimmunoassay technique. T_4 levels decreased by 40–54% after T_3 treatment, whereas T_3 levels rose dramatically (Table 4). On treatment with T_4, both serum T_3 and T_4 increased, the former considerably more dramatically.

DISCUSSION

In the present investigation we examined the influence of thyroid hormones on brain mitochondrial respiration in euthyroid rats. The efficacy of the T_3 and T_4 treatments was evident from the reduced body weights and serum hormone levels (Table 4), which agrees well with earlier observations [12,13,17]. Treatment with excessive doses of thyroid hormones is generally known to result in decreased respiration and uncoupling of oxidative phosphorylation in mitochondria from thyroid-hormone-responsive tissues such as liver, kidney and heart [9,12,13]. As brain is generally considered to be an unresponsive tissue, one might expect a minimal effect of thyroid hormones on its respiratory activity. However, in contrast, the present study shows brain mitochondrial respiration with four different respiratory substrates to be significantly stimulated by the T_3 and T_4 treatments. These effects were observed at all dose levels of T_4 used, whereas only the higher doses of T_3 were effective.

The increased respiratory rates were not closely correlated with mitochondrial cytochrome content (Table 2), but they did appear to be compatible with hormone-induced increases in the primary dehydrogenases (Table 3). Although the dehydrogenase activities declined somewhat at higher doses of T_4, possibly as a result of increased catabolic effects, these levels were still more than adequate to sustain the observed increase in respiratory rates. However, increased dehydrogenase activities cannot be the sole explanation for the observed increase in respiration because

Table 3 Effect of thyroid hormone treatment on dehydrogenase activities in rat brain mitochondria and cytosol

Experimental details are given in the text and Table 1. Enzyme activities are expressed as nmol/min per mg of protein. Reults are given as means ± S.E.M. of eight observations in each group. *$P < 0.001$, compared with the control.

Treatment	Dose (μg/g body weight)	Enzyme activity in mitochondria			Cytosolic malate dehydrogenase activity
		Glutamate dehydrogenase	Malate dehydrogenase	Succinate dehydrogenase	
Control	–	35.2 ± 1.6	463.8 ± 45.8	32.3 ± 1.9	1641.3 ± 125.0
T_3	0.5	70.8 ± 3.7*	647.2 ± 36.3*	73.7 ± 4.5*	1366.6 ± 105.4
T_3	1.0	79.2 ± 8.0*	768.5 ± 37.9*	71.0 ± 2.9*	1405.9 ± 32.1
T_3	1.5	87.6 ± 8.9*	914.2 ± 82.5*	74.5 ± 4.7*	1669.5 ± 107.3
T_4	0.5	89.4 ± 8.4*	1175.6 ± 88.3*	53.7 ± 1.7*	1918.8 ± 119.1
T_4	1.0	68.9 ± 5.5*	750.3 ± 24.8*	52.2 ± 1.9*	1802.7 ± 108.7
T_4	1.5	54.4 ± 3.3*	635.4 ± 39.6*	54.3 ± 1.2*	1837.1 ± 127.9

T_4 clearly stimulated State-3 respiration with ascorbate + TMPD, an electron-donor system known not to employ a primary dehydrogenase. Thus a case may be made for an additional direct effect of thyroid hormones on electron-transfer activity of the respiratory system. The precise nature of this effect has not been revealed by the methods used in the present study. However, alteration of the mitochondrial lipid composition is an interesting possibility, as mitochondrial electron transfer is known to be sensitive to lipid content [18,19]. In parallel studies we have shown that both T_3 and T_4 treatments affect brain mitochondrial phospholipid profiles and membrane fluidity (C. S. Bangur, J. L. Howland and S. S. Katyare, unpublished work).

It is interesting to note that mitochondrial but not cytosolic malate dehydrogenase activity was stimulated in the brain by the hormone treatment (Table 3); others [20–23] have reported stimulation of both cytosolic and mitochondrial enzymes in tissues such as liver. Earlier efforts to demonstrate stimulation of malate dehydrogenase activity in the brain were unsuccessful [9,21,22], which supported the notion that the brain was an unresponsive tissue [9]. However, in most of these studies, the enzyme activity was measured in whole-tissue homogenates or in cytosol [9,21,22], which might have masked the mitochondrial effect reported here.

It is true that the stimulation of mitochondrial malate dehydrogenase activity in the brain was only 2-fold compared with up to 15-fold increases in tissues such as liver [20–23]. This could possibly be related to the predominance of the thyroid hormone receptor isoform α_2 over α_1 and β_1 in the brain [24]. It is believed that α_1 receptors are important in early brain differentiation, whereas β_1 receptors may be more involved in the postnatal aspect of brain development [24]. After brain development is complete, the α_2 form predominates. The high levels of the α_2 variant, which is less sensitive to thyroid hormone action, may account for the apparent lack of response to thyroid hormone unless very high concentrations of T_3/T_4 in the serum are reached.

Indeed, such an assumption is also supported by the data in Table 4. T_3 treatment suppressed serum T_4 levels and the effects were only manifested when serum T_3 levels reached a value in the range of 2000 ng/dl. In contrast for T_4, higher than physiological concentrations (in μg/dl) were reached at the lowest dose of T_4. Thus clearly the hormone effects were discernible only when higher than physiological concentrations of T_4 and T_3 (exceeding 1500 ng/dl) were reached. This is in excellent agreement with the insensitivity of the α_2 receptor isoform referred to above [24].

In conclusion, the present study shows unambiguously for the first time that thyroid hormones stimulate mitochondrial respiration and dehydrogenases in the adult rat brain, a tissue formerly thought to be unresponsive to thyroid hormone. These results are consistent with our earlier report of stimulation of cerebral respiration with a variety of substrates in neonatal and 11-day-old euthyroid and hypothyroid rat pups [8].

Received 20 January 1994/12 April 1994; accepted 15 April 1994

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