Thyroid hormone treatment alters phospholipid composition and membrane fluidity of rat brain mitochondria

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We examined effects of graded doses of thyroid hormones 3,3',5-tri-iodo-L-thyronine (T₃) and L-thyroxine (T₄) on the lipid composition of rat brain mitochondria. Neither hormone significantly affected the mitochondrial cholesterol or total phospholipid content, but did increase phosphatidylethanolamine (PE) at the expense of phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylcholine (PC). The phosphatidic acid (PA) content was also elevated, suggesting enhanced phospholipid turnover. Changes in sphingomyelin (SPM) and diphosphatidylglycerol (DPG) were minimal. Mitochondrial membrane fluidity also increased after thyroid-hormone treatment, and the increase was closely correlated with PC/PE and SPM/PE molar ratios.

INTRODUCTION

The effects of thyroid hormones on mitochondrial function in tissues such as liver, kidney, skeletal muscle, etc. are well established [1]. Deficiency generally results in decreased respiratory activities; these are restored by thyroid-hormone treatment [1]. Hulbert [2] suggested that a mechanism underlying the modulation of mitochondrial enzyme activities might be altered lipid profiles. Several mitochondrial membrane-bound enzymes are lipid-dependent [3,4], and Chen and Hoch [5,6] reported a partial correlation of activity with the fatty acid composition of phospholipids from inner-membrane vesicles of rat liver mitochondria.

Brain is generally considered to be non-responsive to thyroid hormone, as far as stimulation and dehydrogenase activities are concerned [7]. However, we have recently observed that respiration and malate (and other) dehydrogenases are significantly stimulated in brain mitochondria after treatment in vivo of euthyroid animals with 3,3',5-tri-iodo-L-thyronine (T₃) or L-thyroxine (T₄). It is therefore of interest to find whether thyroid-hormone treatment can also influence phospholipid profiles and membrane fluidity of brain mitochondria.

Upon examining effects of T₃ and T₄ on brain mitochondrial lipids, we find dose-dependent changes in the several phospholipid classes. This paper reports these changes, as well as associated increases in membrane fluidity as measured by fluorescence polarization.

MATERIALS AND METHODS

Animals and isolation of mitochondria

Male albino rats of the Charles-Foster strain weighing between 200 and 250 g were injected for 4 consecutive days with 0.5–1.0 μg of either T₃ or T₄/g body wt. [8,9]. Hormone solutions were prepared daily in 0.9 % NaCl containing 5 mM NaOH, with hormone concentrations such that each animal received 1 μl of the solution per g body wt. Control animals received only the vehicle. Mitochondria were isolated from 10 % (w/v) brain homogenates prepared in 0.32 M sucrose as described previously [8,10]. Mitochondrial pellets were washed three times and finally suspended in the same medium to yield a protein concentration of about 7–8 mg/ml.

The mitochondrial isolation procedure, which was based on that of Ozawa et al. [11], was chosen to minimize contamination by extra-mitochondrial membranes such as synaptosomes. To this end, the procedure included a series of three alternating low-speed (5000 g) and high-speed (10000 g) washes, with removal of any loosely packed pellet from the low-speed sedimentation. Mitochondrial purity was monitored by using criteria described by Lai and Clark [12]. Thus, the specific activity of acetylcholinesterase, a marker of synaptosome membranes, in the brain mitochondria was 0.36±0.02 nmol/min per mg, or less than 0.1 % of that of pure synaptosomes (which was 378.0±36.6). The specific activity of lactate dehydrogenase, a cytosolic marker, was 0.015 nmol/min per mg in mitochondria, compared with 10.0±0.8 in cytosol (0.15 %). Finally, NADPH–cytochrome c reductase, a microsomal marker, was not detected in the mitochondrial preparation.

Lipid extraction and analysis

Samples of mitochondrial suspensions containing a total of 8–10 mg of protein were extracted twice with chloroform: methanol (3:1, v/v), and pooled extracts were washed with 0.1 vol. of 0.017 % MgCl₂ [13,14]. Measured samples were used for total phospholipid [15] and cholesterol [16] analysis. Two-dimensional t.l.c. was carried out as described previously [17,18].

Fluorescence polarization

Fluidity measurements were carried out on mitochondria in 0.32 M sucrose containing 10 mM Tris/HCl, pH 7.4. The probe was 1,6-diphenylhexa-1,3,5-triene (DPH), which was dissolved in tetrahydrofuran and stored refrigerated in an amber bottle. For fluorescence measurements, mitochondrial samples were resuspended in the buffered sucrose medium to a final protein concentration of 0.2 mg/ml, and the stock DPH solution was added so that the molar ratio of probe to lipid was 1:200 to 1:300 [19]. The mixture was vortex-mixed vigorously and left in the dark for 30 min to permit equilibration of the probe into the membranes.

Fluorescence polarization was measured at 25 °C in a

Abbreviations used: PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; PA, phosphatidic acid; SPM, sphingomyelin; DPG, diphosphatidylglycerol; DPH, 1,6-diphenylhexa-1,3,5-triene; T₃, 3,3',5-tri-iodo-L-thyronine; T₄, L-thyroxine.

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Shimadzu R-5000 spectrofluorimeter containing a program for calculating and printing out fluorescence polarization values, \( P \). Excitation and emission wavelengths were 360 and 430 nm respectively; bandwidths were 5 and 10 mm respectively. Data were accumulated for 5 s for each polarization setting, vertical (parallel) and horizontal (perpendicular); measurements were performed in triplicate for each individual sample [19].

Calculations of fluorescence anisotropy (\( r \)), limited hindered anisotropy (\( r_a \)) and order parameter, \( S \), as described in [18]. The former two components are used to assess the “static component of fluidity” [20].

**Protein assay**

Protein was measured by the method of Lowry et al. [21].

**Materials**

The hormones \( T_3 \) and \( T_4 \), DPH, and phospholipid standards were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Silica gel G was from E. Merck, Darmstadt, Germany. All other chemicals were of the highest purity available.

**RESULTS**

Treatment with \( T_3 \) or \( T_4 \) produced little effect on total phospholipid or cholesterol content of brain mitochondria, although the lowest dose of \( T_3 \) did produce a modest increase (15–17%) in both phospholipids and cholesterol (see Table 1). However, significant changes in individual phospholipid composition were observed (Table 2). Treatment with \( T_3 \) caused a significant increase in phosphatidic acid (PA), while, at the same time, phosphatidyserine (PS) and phosphatidylinositol (PI) decreased by 19–41%, depending on dose. An apparent decreasing trend in sphingomyelin (SPM) was, however, not significant, whereas phosphatidylcholine (PC) decreased significantly (by 7–14%). This was mirrored approximately by increasing phosphatidylethanolamine (PE), whereas changes produced by \( T_4 \) in di-phosphatidylglycerol (DPG) were generally not significant.

Treatment with \( T_3 \) produced similar, but generally more pronounced, effects. Not only were changes of greater magnitude in most cases, but all doses produced significant alteration in both PC and PE. As with \( T_3 \) treatment, DPG was again unaffected.

Since hormone treatment clearly influenced phospholipid composition, we undertook a study of membrane fluidity, as disclosed by fluorescence polarization and anisotropy. Results are presented in Table 3, which shows that increased fluidity is the rule in all cases except in those where animals received only 0.5 \( \mu g \) of \( T_3 \). This treatment also produced the smallest effects on phospholipid composition (Table 2), as well the slightest stimulation of coupled mitochondrial respiration [8]. All other hormone treatments led to decreased values for \( r_a \) and \( S \), indicating increased membrane fluidity.

Table 1 presents molar ratios of cholesterol/phospholipids, PC/PE, SPM/PC and SPM/PE, and represents an effort to correlate observed fluorescence changes with accepted

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of thyroid-hormone treatment on total phospholipid and cholesterol contents in rat brain mitochondria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
<td><strong>Total phospholipid (mg/g) total</strong></td>
</tr>
<tr>
<td>Control</td>
<td>413.8 ± 15.9</td>
</tr>
<tr>
<td>( T_3 ) (0.5 ( \mu g ))</td>
<td>447.7 ± 8.2 ns</td>
</tr>
<tr>
<td>( T_3 ) (1.0 ( \mu g ))</td>
<td>427.3 ± 3.0 ns</td>
</tr>
<tr>
<td>( T_4 ) (0.5 ( \mu g ))</td>
<td>439.2 ± 6.8 ns</td>
</tr>
<tr>
<td>( T_4 ) (1.0 ( \mu g ))</td>
<td>466.0 ± 8.9*</td>
</tr>
<tr>
<td>( T_4 ) (1.5 ( \mu g ))</td>
<td>432.0 ± 15.0 ns</td>
</tr>
<tr>
<td>( T_4 ) (1.5 ( \mu g ))</td>
<td>422.4 ± 14.0 ns</td>
</tr>
</tbody>
</table>

**Materials**

The hormones \( T_3 \) and \( T_4 \), DPH, and phospholipid standards were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Silica gel G was from E. Merck, Darmstadt, Germany. All other chemicals were of the highest purity available.
Table 4  Effects of thyroid-hormone treatment on lipid compositional parameters of rat brain mitochondria

The experimental details are given in the text and in Table 1. Results are given as means ± S.E.M. of duplicate experiments performed with five independent mitochondrial preparations; *P < 0.02, **P < 0.01, ***P < 0.001; ns, not significant.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cholesterol</th>
<th>PC</th>
<th>SPM</th>
<th>SPM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phospholipid</td>
<td>PE</td>
<td>PC</td>
<td>PE</td>
</tr>
<tr>
<td>Control</td>
<td>0.386 ± 0.015</td>
<td>1.272 ± 0.927</td>
<td>0.183 ± 0.008</td>
<td>0.233 ± 0.012</td>
</tr>
<tr>
<td>T3 (0.5 µg)</td>
<td>0.385 ± 0.009 ns</td>
<td>1.043 ± 0.069**</td>
<td>0.175 ± 0.008 ns</td>
<td>0.182 ± 0.008**</td>
</tr>
<tr>
<td>T3 (1.0 µg)</td>
<td>0.370 ± 0.017 ns</td>
<td>0.994 ± 0.103*</td>
<td>0.172 ± 0.006 ns</td>
<td>0.170 ± 0.013**</td>
</tr>
<tr>
<td>T3 (1.5 µg)</td>
<td>0.379 ± 0.009 ns</td>
<td>0.850 ± 0.014***</td>
<td>0.178 ± 0.009 ns</td>
<td>0.151 ± 0.009***</td>
</tr>
<tr>
<td>T4 (0.5 µg)</td>
<td>0.378 ± 0.009 ns</td>
<td>0.953 ± 0.043***</td>
<td>0.171 ± 0.009 ns</td>
<td>0.165 ± 0.015***</td>
</tr>
<tr>
<td>T4 (1.0 µg)</td>
<td>0.363 ± 0.015 ns</td>
<td>0.864 ± 0.027***</td>
<td>0.169 ± 0.005 ns</td>
<td>0.146 ± 0.005***</td>
</tr>
<tr>
<td>T4 (1.5 µg)</td>
<td>0.408 ± 0.011 ns</td>
<td>0.801 ± 0.025***</td>
<td>0.169 ± 0.005 ns</td>
<td>0.137 ± 0.015***</td>
</tr>
</tbody>
</table>

Figure 1  Relationship between fluorescence anisotropy and mitochondrial phospholipid ratios: (a) PC/PE; (b) SPM/PE

Regression coefficients are denoted as $r$.

There are significant changes in the SPM/PC ratio. The PC/PE and SPM/PE ratios did, however, show significant changes; these were plotted versus fluorescence anisotropy, as shown in Figure 1. There are evident correlations between anisotropy, on the one hand, and either PC/PC or SPM/PE, on the other, with regression coefficients of 0.811 and 0.741 respectively. No such correlation could be obtained for cholesterol/total phospholipids or SPM/PC ratios (results not shown).

**DISCUSSION**

The present investigation, a consideration of effects of thyroid hormones on brain mitochondrial phospholipids, was based on our recent observations of alterations by thyroid hormone of rat brain mitochondrial respiration [8] and on the discovery that these hormones influence the lipid composition of discrete brain regions [14,17]. Our data on lipid composition of brain mitochondria from euthyroid controls agree, in general, with reported values [23]. Although total lipid or cholesterol levels did not appear to change significantly after hormone treatment (Table 1), individual phospholipids did, with generally decreasing amounts of PC, PS + PI, and (especially with T4) SPM. In contrast, PE and PA showed significant increases. It should be mentioned that Ruggiero et al. [24] similarly reported decreased PC and increased PE in liver mitochondria from hypothyroid rats. Thyroid hormones therefore seem to regulate mitochondrial PC and PE levels.

These observed changes in phospholipid profiles were reflected in those of membrane fluidity, as measured by fluorescence anisotropy, which exhibited particularly good correlation with PC/PE and SPM/PE ratios (see Table 4 and Figure 1). Although molar ratios of cholesterol/total phospholipid and SPM/PC have been identified as determinants of membrane fluidity [22], in this instance they do not appear relevant. On the other hand, the SPM/PE ratio is well correlated with apparent fluidity, reflecting perhaps the influence of the positive charge on the choline in both PC and SPM.

Thyroid-hormone treatment brought about a significant increase in the PA content of brain mitochondria. Since PA is an important precursor in phospholipid synthesis, its increase suggests increased phospholipid turnover under the influence of thyroid hormones. Similarly, decreased PS + PI might be related to turnover, in the sense that PS can be decarboxylated to yield phospholipid determinants of membrane fluidity [22]. As expected from data in Table 1, the cholesterol/total phospholipid ratio was not altered by any of the hormone treatments, nor were
PE (which we found to increase), and that PI hydrolysis is often a feature of stimulated phospholipid turnover [25]. In this connection, it should be mentioned that thyroid hormones have been reported to promote inositol phospholipid hydrolysis in rat hypothalamus [26]. These and other possible mechanisms for changes in PC and PE content exist, and remain to be explored. Examples include base-exchange reactions, which are well known to occur in mitochondria [25].

Previously, Chen and Hoch [5,6] and Ruggiero et al. [24] reported changes in fatty acid composition of rat liver mitochondrial phospholipids associated with hypo- and hyper-thyroidism. In general, the former resulted in increased saturation, and the latter in increased unsaturation, especially in PE [24]. In the present study, we did not explore fatty acid compositional changes, but the increased PE that we observed would be expected to be associated with an increase in the unsaturation index, and thus increased fluidity (see Table 4 and Figure 1).

In summary, our present study shows that thyroid hormones influence phospholipid metabolism, and thus membrane fluidity, in rat brain mitochondria. This finding should be considered in light of our observation of hormone-induced changes in mitochondrial respiration in brain (see [8]). Although an indirect influence of these hormones on brain cannot at present be excluded, it appears to us that this tissue should be provisionally added to the list of those already known to be under thyroid-hormone regulation.

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