The Influence of Thyroxine Administered in vivo on the Transmembrane Protonic Electrochemical Potential Difference in Rat Liver Mitochondria

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When mitochondria from normal and thyroxine-treated rats were energized by incubation with succinate, phosphate and MgCl₂, it was found that the hormone treatment increased the transmembrane protonic electrochemical potential difference by 16 mV and the respiration rate by 46%. Other experiments show these changes to be associated with increases in the intramitochondrial K⁺ and phosphate concentrations.

Mitochondria isolated from normal and hypothyroid rats within 24h of treatment of the animals with thyroid hormones have elevated respiratory rates which are not associated with comparable increases in the amounts of respiratory-chain constituents (Bronk, 1963, 1966). Moreover, no decrease in phosphorylation efficiency is seen (Bronk & Bronk, 1961; Bronk, 1963; Hoch, 1968), so that, if the aerobic mitochondrial transmembrane protonic electrochemical potential difference (Δp) forms a link between electron transport and ATP synthesis (Mitchell, 1968, 1976; Papa, 1975), a thyroxine-mediated decrease in Δp appears unlikely. Most factors that stimulate respiratory rate in mitochondria decrease the Δp (Mitchell & Moyle, 1968; Azzone et al., 1978). However, Nicholls (1974, 1977) has reported that the mitochondrial respiratory rate increases dramatically when Δp exceeds a critical value. We provide evidence in this communication that the effect of thyroxine upon mitochondria may involve such a phenomenon.

Methods

Male Wistar rats weighing between 200 and 250g were paired; for each experiment one member of each pair was injected subcutaneously with 800mg of thyroxine (Sigma Chemical Co., Kingston upon Thames, Surrey, U.K.)/kg body weight, and the other with an equivalent volume of iso-osmotic NaCl. After 24h liver mitochondria were isolated (Kielley & Kielley, 1951). Protein was determined by a biuret method (Layne, 1967).

Abbreviations used: Δp, protonic electrochemical potential difference; ΔE, electrical potential difference across the mitochondrial inner membrane; ΔpH, transmembrane pH difference.

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Measurements of Δp

These were carried out by combining and adapting established techniques (Mitchell & Moyle, 1968; Nicholls, 1974, 1977). Mitochondria (4mg of protein/ml) were incubated at 30°C in well-oxygenated media containing 120mM-sucrose, 10mM-Tris/phosphate, pH 7.0, 5mM-MgCl₂, 5mM-Tris/succinate, 0.1mM-KCl, 0.01mM-sodium acetate and 100ng of valinomycin (Sigma Chemical Co., Kingston upon Thames, Surrey, U.K.)/mg of protein in a total volume of 7.75ml. This was supplemented with either (a) [¹⁴C]sucrose (0.1µCi/ml) and [³H₂O (0.2µCi/ml) in order to determine matrix volume (Heaton & Nicholls, 1976) or (b) sodium [³H]acetate (0.2µCi/ml) to determine the ΔpH from the transmembrane acetate distribution (Klingenber, 1970). Samples (0.5ml) were taken as required and the mitochondria were separated immediately by centrifugation (10000g) through a silicone layer (Harris & van Dam, 1968) into 2M-HClO₄. Medium K⁺ was monitored continuously with a suitably calibrated K⁺-sensitive electrode (Mitchell & Moyle, 1968). After assay of K⁺ in the HClO₄ (Amoore et al., 1958) and correction for mitochondrial volume, the matrix K⁺ content was computed. The volume of non-solvent water was taken to be 0.3µl/mg of protein. Respiratory rates were measured with a Rank oxygen electrode, corrected as described previously (Bronk & Parsons, 1965).

Ion accumulation

Mitochondria (4mg of protein/ml) were incubated at 30°C in well-oxygenated media containing 80mM-sucrose, 20mM-KCl, 10mM-Tris/phosphate, pH 7.0, 5mM-Tris/succinate, 5mM-MgCl₂ plus [¹⁴C]sucrose and [³H₂O as above. In addition to K⁺ determination, samples were taken for phosphate analysis (Murphy
& Riley, 1962) and Mg\(^{2+}\) assay with an EIL 351 atomic absorption spectrophotometer.

**Radioisotope assay**

Radioactive samples were taken into Patterson & Green's (1965) solution; a correction was made for spill-over during dual-isotope counting (Koch, 1968).

**Results and Discussion**

The medium employed to determine \(\Delta p\) was chosen because it gave the necessary increased K\(^+\) permeability, although the mitochondrial respiratory rate was not affected by the presence or absence of valinomycin for at least 5 min. This is an essential prerequisite if the effects of thyroxine-stimulated respiration on \(\Delta p\) itself are to be investigated.

Table 1 demonstrates that there was a 13 mV increase in \(\Delta E\) in mitochondria from hyperthyroid rats under State-4 incubation conditions (Chance & Williams, 1955). This difference is associated with a factor of 1.6 in the concentration ratio of K\(^+\) between matrix and medium in the two populations of mitochondria, and can therefore be considered to be outside the experimental errors involved in determining \(\Delta E\). The \(\Delta E\) is a large proportion of \(\Delta p\) because the high phosphate concentration would be expected to convert most of the \(\Delta \text{pH}\) to \(\Delta E\) by phosphate/OH\(^-\) antiport. The difference in \(\Delta E\) values contributes most of the 16 mV increase in \(\Delta p\) which is caused by thyroxine treatment, and these changes are associated with a respiratory stimulation of about 46%. Our \(\Delta p\) determinations for control mitochondria in State 4 are in excellent agreement with data published elsewhere (Azzone et al., 1978), which give a range of about 180–190 mV. However, other observations of \(\Delta p\) vary from 148 mV (Padan & Rottenberg, 1973) to near 230 mV (Mitchell & Moyle, 1968; Nicholls, 1974), and the differences are probably due to the variety in incubation conditions and analytical procedures that have been used. The \(\Delta p\) differences we observed are reproducible and the differences between control and treated values are significant as measured by Student's \(t\) test \((P<0.01)\), but nevertheless we sought independent evidence that these differences were important physiologically.

The matrical accumulation of K\(^+\) and phosphate is driven by \(\Delta p\) (Brierley, 1976), and Table 2 demonstrates the ability of mitochondria from hyperthyroid animals to retain greater quantities of these ions compared with control mitochondria \((P<0.001)\).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(\Delta E) (mV)</th>
<th>(-60\ \Delta \text{pH}) (mV)</th>
<th>(\Delta p) (i.e. (\Delta E-60\ \Delta \text{pH})) (mV)</th>
<th>Respiratory rate (ng-atoms O/min per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>181 ± 4.04 (5)</td>
<td>14 ± 3.8 (5)</td>
<td>195 ± 5.5 (5)</td>
<td>20.4 ± 1.7 (8)</td>
</tr>
<tr>
<td>Thyroxine-treated</td>
<td>194 ± 5.3 (5)</td>
<td>17 ± 4.1 (5)</td>
<td>211 ± 5.9 (5)</td>
<td>29.7 ± 2.3 (8)</td>
</tr>
<tr>
<td>Control + ADP</td>
<td>103 ± 3.8 (4)</td>
<td>10 ± 1.8 (4)</td>
<td>113 ± 4.2 (4)</td>
<td>105.6 ± 2.7 (8)</td>
</tr>
<tr>
<td>Thyroxine-treated + ADP</td>
<td>105 ± 5.8 (4)</td>
<td>8 ± 1.6 (4)</td>
<td>113 ± 6 (4)</td>
<td>140.8 ± 11.0 (8)</td>
</tr>
</tbody>
</table>

**Table 1. Comparison of \(\Delta p\) and respiratory rates in normal rat liver mitochondria with those from the livers of hyperthyroid animals**

Incubations were as described in the Methods section, except where State 3 was initiated by adding 300 nmol of ADP/mg of protein. \(\Delta p\) was recorded 3–5 min after adding the mitochondria, except in the presence of ADP, when \(\Delta p\) measurements were made within 1 min. Mean standard errors are quoted for the number of paired preparations shown in parentheses. The control respiratory rates (+ADP) are significantly different \((P<0.001)\) from those of the treated mitochondria. \(\Delta p\) differences in State 4 are significantly greater in thyroxine-treated mitochondria \((P<0.01)\).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Matrix volume ((\mu l) of water/mg of protein)</th>
<th>Ion content (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mg(^{2+})</td>
</tr>
<tr>
<td>Control</td>
<td>1.49 ± 0.15 (5)</td>
<td>35.6 ± 3.3 (2)</td>
</tr>
<tr>
<td>Thyroxine-treated</td>
<td>1.34 ± 0.11 (5)</td>
<td>37 ± 1.8 (2)</td>
</tr>
</tbody>
</table>

\((P>0.05)\)

**Table 2. Influence of thyroxine treatment in vivo on matrix volume and ion content of rat liver mitochondria incubated for 15 min**

Mitochondria were incubated as described in the Methods section. Matrix volumes exclude non-solvent water. Means ± S.E.M. are given for the numbers of paired preparations shown in parentheses. The probabilities given in the bottom line indicate the significance of the difference between control and thyroxine-treated mitochondria.
when they are incubated without valinomycin. Table 2 also illustrates that there is no increased affinity for Mg\(^{2+}\) at the expense of K\(^+\). These differences in ion content in State 4 would be expected if the thyroxine-treated mitochondria possessed a larger \(\Delta \rho\).

Our results provide the first demonstration that thyroxine-stimulated respiratory rate in mitochondria is associated with an elevated \(\Delta \rho\) in State 4. Hence the physiological effect of thyroxine cannot, as some still maintain (Marzoev & Vladimirov, 1977), be a result of uncoupling of oxidative phosphorylation, which results in a decrease in \(\Delta \rho\) (Mitchell, 1968).

Thus we propose that thyroxine elevates \(\Delta \rho\) to the point at which Nicholls (1974) suggests that some change in the membrane occurs, causing a marked increase in the conductance of the membrane to protons. The electron-transport chain could then operate more rapidly without any decrease in the efficiency of ATP synthesis. Our results indicate that thyroxine stimulates respiration when \(\Delta \rho\) is about 20 mV below the value at which Nicholls (1974) found increased proton conductance and may indicate that the hormone influences membrane permeability as well as \(\Delta \rho\).

At this point we can only speculate on possible mechanisms for the thyroxine effect, but there is evidence for a direct interaction between thyroxine and the membrane in the results of Sterling et al. (1977), which describe specific high-affinity thyroxine-binding sites in the inner mitochondrial membrane. If the increase in \(\Delta \rho\) requires modification of the membrane's electrical characteristics, this might involve the thyroxine-induced decrease in the unsaturated fatty acid content of the membrane, reported by Hulbert et al. (1976) to occur within the treatment time employed here. Hulbert (1978) suggested that this effect was a consequence of the deiodination of thyroxine via the formation of lipid peroxidases.

Since the \(\Delta \rho\) values in our two populations of mitochondria are identical during the phosphorylation of ADP, although the respiratory rates are faster in thyroxine-treated mitochondria, the mechanism of hormonal stimulation in State 3 (Chance & Williams, 1955) may be more complex. It is conceivable that the change in fatty acid content of the membrane (Hulbert et al., 1976) may alter the activity of one or more of the enzymes involved in ATP synthesis, and in this respect, thyroxine-effected elevation of ADP transport (Babior et al., 1973) could be important. However, the increased phosphate concentration in thyroxine-treated mitochondria (Table 2) suggests another possible explanation for the hormonal stimulation of State-3 respiration, namely that the effective \(\Delta \rho\) required for ATP synthesis would be lower in the treated mitochondria.

The most important feature of our findings is that they lead to the formulation of a hypothesis for the mechanism responsible for stimulation of State-4 respiration by the thyroid hormones. We believe that an understanding of this mechanism may bring us closer to determining whether the interaction of thyroxine with mitochondria is responsible for the regulation of basal metabolic rate by the thyroid gland.

References

Mitchell, P. (1968) Chemiosmotic Coupling and Energy Transduction, Glynn Research, Bodmin