The influence of thyroid hormone on the degree of control of oxidative phosphorylation exerted by the adenine nucleotide translocator

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Impaired phosphorylation efficiency in liver mitochondria from hypothyroid rats is paralleled by a defect in adenine nucleotide transport. Both of these lesions can be corrected within 15 min by a near-physiological dose of triiodo-L-thyronine. Measurement of the control strength of the translocator shows, however, that this step has a smaller share of the control for oxidative phosphorylation after thyroidectomy and that this is unaltered after 15 min by replacement therapy. Rapid control by triiodothyronine is thus exerted elsewhere than at this transfer and the effects of hormone on the translocator are likely to be indirect.

Adenine nucleotide translocator *Control strength* *Liver mitochondria* *Oxidative phosphorylation* *Triiodothyronine*

1. INTRODUCTION

Whereas studies on the mechanism of thyroid hormone action have in recent years centred on the control of transcription in cell nuclei (see [1]), there is also evidence for direct rapid effects on mitochondria. Both we [2] the author in [3] have shown that mitochondria acquire hormone preferentially at very short times after administration to thyroidectomised animals and 3 different laboratories have reported the presence of specific tight-binding receptors in mitochondrial membranes [4–6]. In addition, authors in [3,4,7,8] and our laboratory [2,9–11] have provided evidence for rapid effects of physiological levels of L-triiodothyronine (T_3) on energy-driven processes in mitochondria. In particular, we have developed a preparation of liver mitochondria from thyroidectomised rats which shows significantly lower ADP/O ratios with no loss of respiratory control or evidence of uncoupling. Moreover, mitochondria prepared 15 min after intravenous injection of a near-physiological dose of T_3 to these animals have ADP/O ratios indistinguishable from preparations from normal animals, although their rate of oxygen uptake per mg protein remains depressed. That the lowered phosphorylation efficiency is not a preparation artifact is suggested by the finding that whole tissue adenine nucleotide ratios are altered after thyroidectomy to reflect a reduced ability to rephosphorylate ADP and corrected within 15 min of administering hormone [2]. Also, ^{31}P-NMR studies by the saturation transfer method of perfused rat hearts from thyroidectomised rats have shown that the steady-state flux of ATP synthetase is proportionately more impaired than is oxygen consumption [12].

One potential component in this lowered phosphorylating ability may be the reduced rate of the adenine nucleotide translocator reported in hypothyroid mitochondria [10,13,14] since authors in [15–17], applying the theories [18,19], have shown that a significant measure of control of oxidative phosphorylation may be exerted by the transfer. In recent experiments [10] we have found that, although the V_{max} is lowered by only 15%, the apparent K_m of the liver translocator is very significantly increased by thyroidectomy and re-

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stored to normal in mitochondria isolated 15 min after intravenous injection of a near-physiological amount of T_3. Nevertheless, the affinity of the translocator for ADP after thyroidectomy is still relatively high (K_m apparent $\simeq 20$ μM in the presence of Mg^{2+} [10]), while lowered ADP/O ratios are observed at ADP concentrations around 200 μM [2,11,20]. In an attempt to assess the significance for oxidative phosphorylation of the altered translocator characteristics in hypothyroid animals we have used the method of [15] to measure the control strength of this step after thyroidectomy and the effect of T_3 replacement therapy on this.

2. METHODS

2.1. *Animals*

Male albino rats, 130–150 g, bred in the department were thyroidectomised and maintained as previously described [2,11]. Where appropriate they were given 1 nmol/100 g body weight of triido-L-thyronine via the lingual veins 15 min prior to killing.

2.2. *Mitochondria*

Liver mitochondria were prepared in 0.25 M sucrose/1 mM EDTA/10 mM triethanolamine-HCl (pH 7.4) washed once with this buffer, once with 0.25 M sucrose and resuspended at ~40 mg/ml in 0.25 M sucrose. Trace metals were removed from sucrose by passage down a Dowex-50 (Sigma, St. Louis, MO) acid-form column.

2.3. *Oxygen electrode experiments*

Estimation of the ADP/O ratios were as previously described [2,11,20]. Carboxyatractyloside inhibition experiments were carried out at 37°C as described [15] in 1.6 ml of pH 7.4 buffer containing 15 mM KCl, 50 mM Tris-HCl, 5 mM $MgCl_2$, 2 mM EDTA, 2 mM malate, 20 mM glucose, 10 mM potassium phosphate, 20 mM succinate, 1 μg/ml rotenone, 5 mM sucrose, ~1 mg/ml mitochondrial protein and either 1 mM or 33 μM ATP (Tris salt). The rate of respiration was varied by adding differing quantities (~0.3–3 U) of yeast hexokinase (Boehringer, Lewes, England) and 2–30 μl of 8 μM carboxyatractyloside (Sigma, St. Louis). Protein was determined by the method of [21].

3. RESULTS AND DISCUSSION

Fig.1 gives an example of the titration with carboxyatractyloside of a mitochondrial preparation from a thyroidectomised animal adjusted to different rates of respiration between states 3 and 4 by adding hexokinase in the presence of glucose and

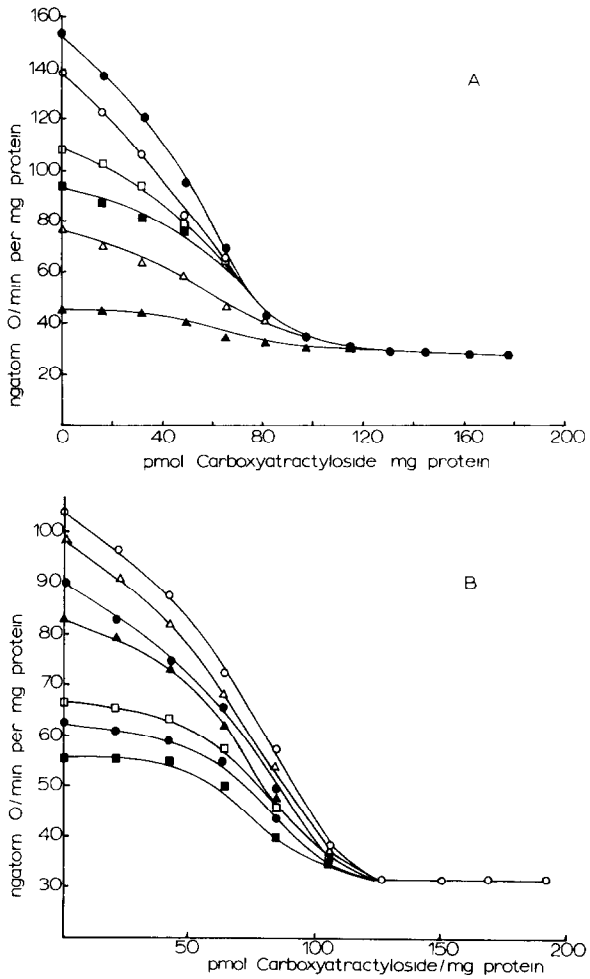

Fig.1. The inhibition by carboxyatractyloside of oxygen uptake by liver mitochondria from (A) a normal rat and (B) from a rat thyroidectomised 6 wk before sacrifice. Different initial steady-state rates of respiration were obtained by adding different amounts of yeast hexokinase to 1.6 ml buffer (see text) containing 33 μM ATP. Different symbols are used to distinguish different amounts of hexokinase. The ADP/O ratio with succinate as substrate was found in separate experiments [2,11]. (A) 1.48 mg mitochondrial protein; ADP/O = 1.97. (B) 1.51 mg mitochondrial protein; ADP/O = 1.01.

ATP. About 8–10 different initial rates were usually chosen though a smaller number is shown here to aid clarity. Authors in [22] have shown that using an irreversible inhibitor such as carboxyatractyloside the control strength or sensitivity [18,19] of the translocator (C) can be calculated from the expression

$$C = -I_{max} \frac{1}{F} (\delta F/\delta I)_{I=0}$$

where I_{max} is the concentration of inhibitor giving maximum inhibition, F represents the steady-state flux (oxygen consumption) and $(\delta F/\delta I)_{I=0}$ is the slope of the F vs I function (fig.1) at zero inhibitor concentration.

Carboxyatractyloside titrations of this kind were carried out with liver mitochondria prepared from both euthyroid animals and from animals thyroidectomised 6 wk before the experiment. Fig.2 presents the calculated control strengths in two such preparations as the respiration was varied from state 4 (relative respiration = zero) to state 3

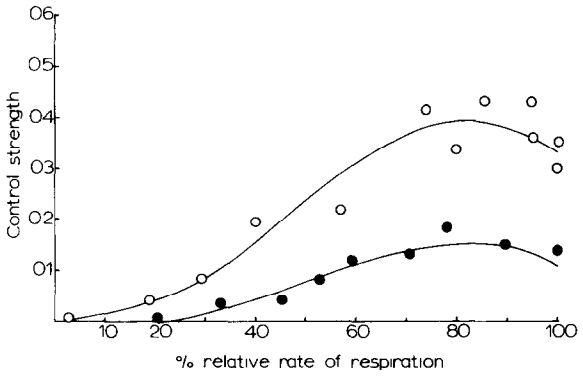

Fig.2. The control strengths of the adenine nucleotide translocator as a function of the relative rates of ADP-stimulated respiration (state 4 = 0; state 3 = 100%) in a mitochondrial preparation from a normal rat (○; ADP/O = 1.97; 0.93 mg protein/ml; I_{max} = 0.11 nmol/mg protein; state 3(4) respiration = 183(18(ngatom O/min per mg protein and from a rat thyroidectomised 6 wk prior to experiment (●; ADP/O = 1.3; 0.90 mg protein/ml; I_{max} = 0.12 nmol/mg protein; state 3(4) respiration = 150(36) ngatom O/min per mg protein. The carboxyatractyloside titration was carried out as described in section 2 and in the legend to fig.1; the initial ATP concentration was 1 mM.

(100%). The control strengths of the translocator at all levels of stimulated respiration are lower after thyroidectomy. In a series of such experiments where 1 mM ATP was added, the mean control strength at 80% relative respiration was found to be 0.306 ± 0.043 (SE; $n = 5$) for euthyroid preparations and significantly lower ($p < 0.025$) for hypothyroid mitochondria at 0.17 ± 0.013 ($n = 5$). The mean ADP/O ratio with succinate as substrate were respectively 1.81 ± 0.06 and 1.22 ± 0.09 (t-test; $p < 0.0005$). A control strength of about 0.3 for normal mitochondria agrees well with the values found at around 80% relative respiration under identical conditions [15,22]. The lower control strengths found for the translocator after thyroidectomy show that this step has a smaller share of the overall control than in normal animals; and, since the sum of the control strengths for all the steps in oxidative phosphorylation under steady-state conditions is unity [18,19], that a greater share of the control must correspondingly lie elsewhere.

Using glucose, yeast hexokinase and 10 mM phosphate, state 3 respiration is attained at an ATP/ADP ratio of around 5 [23,24]. This implies that at 1 mM total adenine nucleotide, the ADP concentration at state 3 may be about 165 μM. Under the conditions of this experiment (5 mM Mg^{2+}, 2 mM EDTA, 15 mM K^+) most of the ADP would be complexed with Mg^{2+} [25,26]. Thus, near state 3, the concentration of magnesium–ADP complexes is well above the apparent K_m of the translocator for this substrate in either euthyroid or hypothyroid liver [27,10]. Hence, we also examined the control strengths at a total adenine nucleotide concentration of 33 μM. Fig.3 shows that while the absolute values of the control stengths have increased, those of the hypothyroid preparation are still lower than normal. At 80% relative rate of respiration the values found were 0.64 ± 0.01 ($n = 3$) and 0.30 ± 0.04 [4] for normal and hypothyroid preparations respectively. The higher control strengths are presumably a reflection of the connectivity theorem [18,24] and imply that at the lower ATP concentration the elasticity of the yeast hexokinase (K_m apparent for ATP = 200 μM [28]) is increased relative to that of the transporter. Fig.3 also demonstrates that i.v. injection of triiodo-L-thyronine 15 min before killing did not alter the measured control strength. In 3

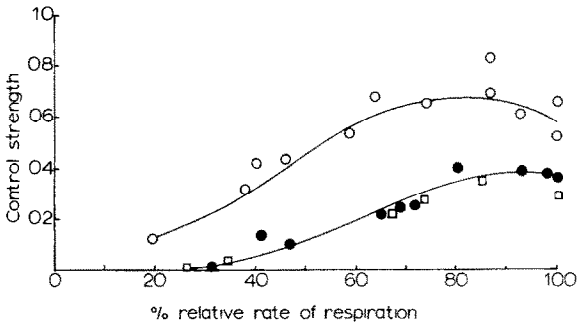

Fig.3. The control strengths of the adenine nucleotide translocator as a function of the relative rate of respiration as in fig.2 except that the initial ATP concentration was 33 μM. The euthyroid (○) and hypothyroid results (●) were calculated from the data shown in fig.1. The control strengths of mitochondria prepared from a thyroidectomised rat given 1 nmol L-triiodothyronine per 100 g body weight via the lingual veins 15 min before sacrifice are also shown (□), in this case I_{max} = 0.12 nmol/mg protein, ADP/O ratio = 1.94, and the protein concentration was 1.25 mg/ml and state 3(4) respiration was 102(31) ngatom/min/mg

experiments the control strength at 80% relative respiration was 0.25 ± 0.04 whereas the ADP/O ratio with succinate was indistinguishable from normals at 1.81 ± 0.18.

4. CONCLUSION

Although the K_m apparent of the adenine nucleotide translocator is significantly increased by thyroidectomy [10] and very rapidly restored by hormone replacement, the measure of control of oxidative phosphorylation exerted by the transporter in hypothyroid preparations is significantly lower than in normals. Thyroidectomy thus leads to an increase in control at another step or steps which must therefore be considered the principal direct sites for rapid hormone action in preference to the translocator. That replacement therapy does not increase the control strength of the transporter within 15 min suggests that this may be dictated mainly by the relative quantities of translocator protein and of other enzymes present in these preparations.

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