Communication

Rapid Effects of a Subcalorigenic Dose of 1-Thyroxine on Mitochondria*

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SUMMARY

2,4-Dinitrophenol raised the basal metabolic rate of O2 consumption less in some hypothyroid rats (group A) than in others (group B) which responded like normal rats. A single, subcalorigenic dose of 1-thyroxine, 5.2 ng per g, partly restored this response in basal metabolic rate in 3 hours in rats of group A. The function of their liver mitochondria differed according to the previous responses in vivo of the rats to dinitrophenol, and according to the degree of calorigenesis induced in vivo by dinitrophenol in rats pretreated 3 hours with 1-thyroxine. The hormone acted rapidly in vivo on liver mitochondria to regulate their reactivity to dinitrophenol and their respiratory control.

2,4-Dinitrophenol raises the basal metabolic rate of O2 consumption by depressing mitochondrial respiratory control (1). Its calorigenic action is thus a measure of responses of mitochondria in the living cell. Dinitrophenol raised the basal metabolic rate less in some hypothyroid rats (group A) than in others (group B), which responded like normal rats. This difference, as discussed elsewhere (2), may arise because rats from group A are more hypothyroid than those from group B, as judged by the cytochrome c content of their liver mitochondria. A single, subcalorigenic dose of 1-thyroxine, 5.2 ng per g of body weight, restored the response in basal metabolic rate when dinitrophenol was administered to rats of group A to the extent of 60% of normal in 3 hours and 85% in 6 hours. 1-Thyroxine given in vivo therefore increased either the sensitivity of the mitochondria of rats of group A to dinitrophenol or the accessibility of dinitrophenol to reactive sites in the mitochondria. To test the first alternative, the relation between mitochondrial function in vivo and in vitro was examined. Sensitivity to dinitrophenol, as represented by the rate of respiration in State 3U (3), was measured in mitochondria obtained from livers of each group of hypothyroid rats. The response in vitro to dinitrophenol of mitochondria from group A was lower than that in mitochondria from group B, and increased to nearly equal that in rats of group B 3 hours after treatment with 1-thyroxine, 5.2 ng per g. This dose had no effect on mitochondria from rats of group B. Mitochondrial respiratory control was inversely related to dinitrophenol sensitivity, and State 4 respiration was directly related; both changed in mitochondria of rats of group A after treatment with 1-thyroxine.

The function of liver mitochondria differed according to the previous responses in vivo of the rats to dinitrophenol (Table I). Mitochondria from rats of group A oxidized glutamate in State 3U more slowly than did mitochondria from group B, particularly in the presence of cytochrome c and NAD+ (Experiment I of Table I), reflecting the differences in dinitrophenol induced calorigenesis between rats of group A and B in vivo; respiration in State 4 was lower, and respiratory control higher, than in rats of group B. The efficiency of phosphorylation by mitochondria from both groups was the same when measured by the estimation of orthophosphate in the presence of a glucose-hexokinase system.

The function of liver mitochondria also differed according to the degree of calorigenesis induced in vivo by dinitrophenol in rats pretreated 3 hours with 1-T4, 5.2 ng per g. In the rats of group A, L-T4 increased mitochondrial State 3U (only when measured in the presence of cytochrome c and NAD+) and State 4 respiration, decreased respiratory control, and left the phosphorylation quotient unchanged. This rise in State 3U respiration reflected the animals' increased responses in vivo to dinitrophenol 3 to 6 hours after L-T4 was given (2). In the rats of group B, injected L-T4 did not change mitochondrial function in vitro nor did it change the degree to which dinitrophenol raised the basal metabolic rate in vivo. The sensitivity of liver mitochondria toward dinitrophenol added in vitro was consistent with the effects of thyroid administration on the calorigenic action in vivo of dinitrophenol in hypothyroid rats, supporting the alternative posed above that L-T4 acted in vivo on mitochondria to control their reactivity toward dinitrophenol.

Respiratory control (the ratio of respiration in State 4 to State 3) was higher in liver mitochondria obtained from rats of group A than in those of group B; in both, respiratory control was higher than in liver mitochondria from euthyroid rats (4), confirming the results of Maley and Lardy (6) with glutamate as substrate but not those of Bronk and Bronk (7) with succinate or β-hydroxybutyrate. The dose of L-T4 that decreased mitochondrial respiratory control did not raise the basal metabolic rate in vivo (2), and so decreased respiratory control and a calorigenic effect were not necessarily related in these rats, perhaps partly because liver respiration contributes as little as 10% to the basal O2 consumption of starved rats (8). Thyroid status or treatment with L-T4 did not affect the phosphorylation quotient in these experiments.

The hormone used (L-T4), the size of the dose (7 pmoles per g), the speed of action (3 hours), and the intraperitoneal route of administration all combine to show that the most rapid and efficacious effect of thyroid hormone in vivo is on mitochondria. When about 100 times as much L-T4 was administered in 14 daily doses to thyroidectomized rats, the respiratory control of liver mitochondria obtained from such animals declined (9). A single dose of L-triiodothyronine about 3000 times larger than 1 The abbreviation used is: L-T4, L-thyroxine.
TABLE I

Effect of single, subcalorigenic dose of L-thyroxine in 3 hours on function of liver mitochondria from hypothyroid rats

Hypothyroid male rats (thyroidectomized (Charles River Breeding Laboratories, Inc.), treated by injection with 100 I, and fed a low iodine, high vitamin diet) were divided into two groups according to their calorigenic responses to intraperitoneal dinitrophenol, 10 µg per g; group A responding subnormally, and group B normally (2). They were fasted 17 to 24 hours before experiments were performed. One of a pair of animals in each group (9 pairs in A, 8 pairs in B) was given 0.9% NaCl solution by intraperitoneal injection, the other L-thyroxine, 5.2 ng per g, and both were sacrificed 3 hours later. Liver mitochondria were isolated and assayed as in Reference 4, with glutamate as substrate, for respiration (based on protein content (5)) in State 4 (without phosphate-acceptor, hexokinase-glucose), State 3U (with 50 µM dinitrophenol), and State 3 (with phosphate-acceptor); for respiratory control (the ratio of State 4 to State 3); and for efficiency of energy transfer (phosphorylation quotient). In Experiment I, 0.33 mM NADH and 3.3 µM cytochrome c were added to the reaction mixture; in Experiment II, they were omitted. The significance of differences was calculated for series of pairs within each group* and for differences between groups†.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Respiration</th>
<th>Respiratory control</th>
<th>Phosphorylation quotient</th>
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<tr>
<td></td>
<td>State 4</td>
<td>State 3</td>
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</tr>
<tr>
<td></td>
<td>µl O2/hr/mg protein</td>
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<tr>
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<td>51.8</td>
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* p < 0.05 (paired comparisons).
† p < 0.05 (group comparisons).

that of L-T4 used here raised the basal metabolic rate of thyroidectomized rats and uncoupled oxidative phosphorylation in their liver mitochondria in 2 hours (10); a dose 60 to 75 times larger than ours increased mitochondrial (11) and microsomal protein synthesis in 36 hours and nuclear RNA synthesis in 16 hours (12). An intravenous dose 5 times larger potentiated the lipolytic action of epinephrine on adipose tissue in 3 hours (13).

It remains to be shown whether L-T4 in the present studies changed mitochondrial composition or functional state or both. Treatment in vivo with the current dose of L-T4 did not restore the diminished cytochrome c content of the liver mitochondria from hypothyroid rats of group A (14), an significant synthesis of new respiratory assemblies is not likely. However, the observed decrease in respiratory control would increase the rate of generation of ATP, as originally pointed out by Lardy and Maley (15) and re-emphasized later (16), since State 4 respiration is coupled with energy transformation (17), and secondarily would provide increased potential for cellular synthetic processes. This anabolic action of L-T4 is much more potent and rapid than had been shown previously, and it is not restricted to "toxic" doses.

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REFERENCES

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