THE EFFECT OF PHYSIOLOGICAL DOSES OF THYROXINE ON CARRIER-MEDIATED ADP UPTAKE BY LIVER MITOCHONDRIA FROM THYROIDECTOMIZED RATS

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SUMMARY: Carrier-mediated ADP uptake by liver mitochondria from myxedematous thyroidectomized rats was compared with that of mitochondria from thyroidectomized animals rendered euthyroid or hyperthyroid by exogenous thyroxine (T_4). Uptake by mitochondria from thyroidectomized rats treated with 0, 2, 5, and 20 μg T_4/100 g body weight/day for 6 days was 1.24 ± 0.16, 2.26 ± 0.14, 3.82 ± 0.09, and 8.20 ± 0.58 nmoles ADP/min/mg protein respectively (mean ± SE; $p < .001$ for all differences). Uptake by mitochondria from normal rats was similar to that of mitochondria from thyroidectomized rats treated with 2 μg T_4/100 g/d, a physiological replacement dose. These findings provide further evidence that the calorigenic action of thyroid hormone may be mediated by an action on the mitochondrial ADP-ATP carrier.

We recently reported that the rate of carrier-mediated uptake of ADP into liver mitochondria of rats made thyrotoxic by the administration of L-thyroxine (T_4) or L-triiodothyronine exceeded that of euthyroid animals (1). This observation raised the possibility that the calorigenic effect of thyroid hormone may be related to an effect of the hormone on mitochondrial adenosine nucleotide transport. We have now investigated ADP uptake by mitochondria from thyroidectomized myxedematous rats, thyroidectomized animals rendered euthyroid or hyperthyroid by exogenous T_4, and unoperated euthyroid control rats. These studies have provided further evidence supporting a relationship between mitochondrial nucleotide transport and calorigenesis by thyroid hormone.

MATERIALS AND METHODS

Rats (200-300 g males) were obtained from a highly inbred colony

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maintained at St. Elizabeth's Hospital, Boston, Mass. Thyroidectomies were preformed by standard procedures under ether anesthesia. After 4 weeks the thyroidectomized rats were severely myxedematous, with serum T_4 concentrations (2) less than 0.4 μg/100 ml in every case, compared to normal control values of 3.9 ± 0.7 SD μg/100 ml.

T_4 solutions of various concentrations were prepared as previously described (1). Hormone treatment consisted of 6 i.p. injections of T_4 (0.1 ml/100 g body weight/d) administered as a single injection each day for 6 consecutive days. Where no T_4 was administered, the animals received injections of diluent of comparable volume. Animals were killed by a blow on the head 24 hours after the final injection.

The preparation and testing of mitochondria and the measurement of the rate of ADP uptake was carried out as previously described, except that ADP uptake was measured at 2° rather than 6° (1). Experiments were performed using only mitochondria with respiratory control ratios equal to or exceeding 2.0. Protein was measured by the biuret method. Cytochrome aa_3 concentration was determined as described by Rieske (3), except that Triton X-100 (final concentration 1%) was used as the detergent instead of deoxycholate and cholate, and the differences in absorbance were determined from complete spectra taken between 500 and 700 nm on a Cary 118 C recording spectrophotometer at room temperature before and after the addition of potassium ascorbate.

RESULTS

Carrier-mediated uptake of ADP by liver mitochondria from animals of varying thyroid status is presented in Table I. ADP uptake was lowest in the myxedematous animals and rose progressively as the dose of T_4 increased. The difference in ADP uptake between groups of differing thyroid status was highly significant ($p < .001$). The only groups between which the difference was not significant were the two euthyroid groups: the thyroidecto-

TABLE I

Carrier-mediated ADP uptake by liver mitochondria from myxedematous, euthyroid, and hyperthyroid rats

Operation	T_4 (μg/100 g/d)	ADP uptake* (nmoles ADP/min/mg protein)
Thyroidectomy	0[†]	1.24 ± 0.16 (6)
"	2[†]	2.26 ± 0.14 (7)
"	5[†]	3.82 ± 0.09 (7)
"	20[†]	8.20 ± 0.58 (6)
None	0[§]	2.40 ± 0.11 (7)

For details see text. Figures in parentheses represent the number of animals used in each experiment.

* Mean ± SE.

[†] All differences among these groups are highly significant ($p < .001$).

[§] Differences between the non-thyroidectomized control rats and thyroidectomized rats receiving 0, 5, and 20 μg T_4/100 g/d are highly significant ($p < .001$). Differences between the non-thyroidectomized control rats and the thyroidectomized rats receiving 2 μg T_4/100 g/d are not significant.

mized animals given 2 μg of T_4/100 g/d (a physiologic replacement dose (4)) and the unoperated animals given diluent alone.

The data in Table I are all expressed in terms of mitochondrial protein. To determine whether mitochondrial protein reflected the true concentration of mitochondria in the various preparations, the concentration of cytochrome aa_3 was measured in certain of the preparations. This cytochrome complex is a tightly bound component of the inner mitochondrial membrane, and its concentration would be expected to provide an accurate estimate of the concentration of mitochondria in the preparation. Due to problems of storage, it was only possible to measure cytochrome aa_3 in certain of the preparations from myxedematous rats (i.e., thyroidectomized rats which had received only diluent) and from unoperated controls. In 3 preparations from myxedematous animals, the concentration of cytochrome aa_3 was 0.192 ± 0.028 SD nmoles/mg mitochondrial protein, which was not

significantly different from values measured in preparations from 4 normal control rats (0.213 $\pm$ 0.017). Thus there is no significant difference in the quantity of mitochondria per mg protein between the preparations from the normal and myxedematous animals as measured by cytochrome aa_3 concentration.

DISCUSSION

Within each group, the scatter in ADP uptake was much smaller in these experiments than in those reported previously (1). This may be attributable to the homogeneity of the strain of rat used in the present experiments, inbred for many years. The difference in uptake between euthyroid animals and animals given the largest dose of T_4 (20 µg/100 g/d) was substantially larger than previously observed. We have no explanation for this finding.

There is evidence that the carrier-mediated ADP-ATP exchange reaction which is responsible for the uptake of ADP into the mitochondrial matrix (5), the site of oxidative phosphorylation (6), may be the rate-limiting step of mitochondrial energy production (7). Therefore, it would be reasonable to propose that thyroid hormone exerts its effect on oxygen consumption and metabolic rate at least in part by stimulating this exchange reaction. Our results on the effect of physiological doses of thyroid hormone on mitochondrial ADP uptake, particularly those showing differences between mitochondria obtained from myxedematous and euthyroid animals, support this hypothesis.

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