Effects of Thyroid Hormone on Adenine Nucleotide Content of Rat Liver[1] (37615)

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(Introduced by A. White)

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Evidence has been provided that stimulation of respiration (QO_2) by thyroid hormone is mediated to a significant extent by augmentation of energy utilization for active Na^+ transport in liver, kidney, and skeletal muscle (1–2). Previous findings also imply that thyroid hormones may increase ATP production as a result of an increase in the activities of glycolytic and mitochondrial respiratory enzymes (3–6). Thus, thyroid hormones may stimulate ATP production and ATP utilization in parallel; although one or the other pathway may predominate. If activation of the Na^+ pump is the dominant response, the ATP/ADP ratio should be inversely related to the change in QO_2. Alternatively, if ATP production predominates, the ATP/ADP ratio should vary directly (but not necessarily linearly) with the thermogenic response.

[1] Supported in part by U.S. Public Health Service Program Project Grant No. HL-06285 from the National Heart and Lung Institute and the Feigenbaum Fund of the Heggblade-Marguleas Foundation.

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Methods. Male, Sprague–Dawley rats (150–200 g body wt) were maintained on Purina chow, *ad libitum*. The thyroidectomized rats were used 3 to 4 wk postoperatively. Thyroidectomized and euthyroid rats were injected subcutaneously either with L-3,5,3′-triiodothyronine (T_3) (50 μg/100 g body wt) or with a comparable volume of the diluent (5×10^{-4} *M* NaOH) on alternate days and received a total of three doses. In each group, paired experiments were performed simultaneously on T_3 and diluent-injected rats. Two groups of studies were executed in parallel: In one group, oxygen consumption was determined in liver slices in a Warburg respirometer as described previously (1). In the second group, liver was assayed for adenine nucleotide content by the freeze-quench technique. Twenty-four to 48 hr after the third injection, the rats were anesthetized with intraperitoneal Inactin (5-ethyl-5-(1′-methyl propyl)-2-thiobarbituric acid, sodium salt), 8 mg/100 g body wt. Since some barbiturates (*e.g.*, amytal) inhibit mitochondrial oxidative phosphorylation at high concentrations (7), control measurements on the effect of anesthetic doses of Inactin on liver ATP content were also obtained. For this purpose, euthyroid rats injected with T_3 or diluent were anesthetized either with ethyl ether by inhalation or by peritoneal injection of chloral hydrate, 36 mg/100 g body weight. In 4 euthyroid rats anesthesia with ethyl ether or chloral hydrate yielded hepatic ATP values of 2440 ± 150, ADP values of 820 ± 100 (nmoles/g wet wt) and a mean ATP/ADP ratio of 2.98. This

TABLE I. Effect of T_3 on QO_2 of Liver Slices from Thyroidectomized and Euthyroid Rats.[a]

Thyroid status	No. of rats	QO_2[b]	p
Thyroidectomized	9	5.8 ± 0.3	<0.001
Thyroidectomized + T_3	6	12.7 ± 0.8	
Euthyroid	6	8.0 ± 0.3	<0.001
Euthyroid + T_3	6	13.5 ± 0.5	

[a] Rats were injected either with 50 μg T_3/100 g body wt, or diluent, three times on alternate days and the measurements were made 48 hr after the third injection.

[b] QO_2 is expressed as μl/mg dry wt/hr; mean ± SEM. Statistical significance was evaluated by the unpaired Student's t test.

ATP/ADP ratio (2.98) is somewhat lower than that obtained with Inactin anesthesia (cf. Table II). Accordingly, Inactin was used as the standard anesthetic.

The right lobe of the liver was exposed through a midline, abdominal incision, lifted carefully and then clamped vigorously between aluminum blocks (tongs) which had been precooled in liquid nitrogen (8, 9). The frozen plates of liver were immediately excised and the entire assembly was immersed in liquid nitrogen. The extracts were prepared in a cold room at 2–4°, as described previously (9). The tissue protruding from the edges of the metal clamps was trimmed; the frozen plates of liver (~ 1 mm thick) were transferred to a porcelain mortar, which contained liquid nitrogen, and crushed with a pestle. The frozen powder was weighed in tared polyethylene vials and then covered with ice-cold 0.4 N $HClO_4$ in 30% ethanol. The mixture was sonicated, 5 W/sec, in an ice bath, three times for 15 sec intervals, interrupted by 15 sec cooling periods (Branson Sonic Power Co., Micro-tip Model W-140 D). The sonicates were centrifuged for 1 min in an Eppendorf microcentrifuge, the pellets were re-extracted with 0.4 N $HClO_4$ and the supernatants were immediately neutralized with 2 M K_2CO_3–0.5 M triethanolamine HCl. The precipitates were removed by centrifugation. The extracts were either assayed immediately or were frozen quickly, stored at —70° and assayed within 2 days. Nucleotides were determined by enzymatic-optical methods as described previously (9), ATP content using a Cary 14 spectrophotometer, and ADP and AMP in a Gilford-Beckman recording spectrophotometer, at room temperature. Statistical calculations were based on the unpaired Student's t test (10); significance was attributed to $p < 0.05$.

Results. As shown in Table I, injection of T_3, three doses over a 6 day period, resulted in a 119% increase in hepatic QO_2 in thyroidectomized rats and a 69% increase in euthyroid rats. These thermogenic responses are comparable to those described previously (1, 2).

The effect of T_3 in thyroidectomized rats on liver content of adenine nucleotides is summarized in Table II. ATP, ADP and

TABLE II. Adenine Nucleotide Content of Liver of Thyroidectomized and Euthyroid Rats (± T_3).[a]

Thyroid status	ATP	ADP	AMP	(ATP+ADP +AMP)	ATP/ADP
Thyroidectomized	2315 ± 51	777 ± 52	356 ± 33	3448 ± 84	3.18 ± 0.23
Thyroidectomized + T_3	2155 ± 91	753 ± 35	320 ± 24	3261 ± 108	2.96 ± 0.19
Δ	—160	—24	—36	—187	—0.22
p	ns	ns	ns	ns	ns
Euthyroid	2579 ± 103	670 ± 29	320 ± 29	3567 ± 88	4.00 ± 0.27
Euthyroid + T_3	2049 ± 107	897 ± 52	429 ± 48	3376 ± 82	2.48 ± 0.26
Δ	—530	227	109	—191	—1.52
p	<0.005	<0.001	ns	ns	<0.001

[a] Rats were injected with 50 μg T_3/100 g body wt or diluent three times on alternate days and the measurements were made 24 to 48 hr after the third injection. Nucleotide content is expressed as nmoles/g wet wt of tissue; mean ± SEM. $n = 16$ pairs of rats in each group (i.e., total of 32 rats). ns = statistically nonsignificant.

AMP content were, on the average, lower in T_3-treated animals but not of statistical significance. The liver ATP/ADP ratio of the T_3-injected rat was 7% lower than that of the diluent injected controls, but not statistically significant.

In euthyroid rats, T_3 produced similar changes in adenine nucleotide content but of greater magnitude. As shown in Table II, T_3 evoked a 20% fall in ATP, a 34% rise in ADP levels, and the ATP/ADP ratio fell 38%. These changes were statistically highly significant. It should be noted that total adenine nucleotide content (ATP + ADP + AMP) fell slightly but not significantly with T_3.

Discussion. In previous studies, administration of thyroid hormone produced variable declines in cellular content of ATP (11–14). These measurements, however, are open to question in that the tissues were not frozen *in situ* and cellular ATP content falls rapidly with anoxia (15, 16). The present studies, therefore, made use of a rapid-freeze technique applied *in situ* (8).

Thyroid-induced augmentation of energy consumption by the Na^+ pump could result either from (a) primary activation of the Na^+ transport system, in which case the increase in energy consumption for Na^+ transport would stimulate respiration, or (b) primary stimulation of energy production, presumably oxidative phosphorylation, in which case the increase in the affinity of the chemical reaction (*i.e.*, the differences in the sums of the free energies of the reactants and products) that drives the Na^+ pump would elicit the increase in Na^+ transport (1–2). If thyroid hormones stimulate Na^+ transport via a primary pathway (*e.g.*, by inducing Na^+, K^+-ATPase), the ATP/ADP ratio would tend to fall. Alternatively, primary stimulation of coupled oxidative phosphorylation could enhance Na^+ transport by a rise in the ATP/ADP ratio at the pump site. The results indicate that T_3 elicited a fall in hepatic ATP/ADP ratios in both euthyroid and thyroidectomized rats, although the magnitude of the fall was significant only in T_3-treated euthyroid animals (Table II). No support was obtained for the possibility that a shift in adenine nucleotide concentration, in itself, accounts for increased energy consumption by Na^+ pump [mechanism (b)]. The fall in ATP/ADP ratio noted in the T_3-treated euthyroid rats is consistent with the earlier finding of thyroid stimulation of Na^+, K^+-ATPase activity, as predicted by mechanism (a) (2). The interpretation of these data, however, is complicated by the question of heterogeneity in intracellular distribution of the adenine nucleotides.

In isolated mitochondrial preparations, the rate of respiration and the K_m for ADP have been found to be a function of the ATP/ADP ratio of the external medium (17). Qualitatively, a fall in cytoplasmic ATP/ADP ratio stimulates mitochondrial respiration (7). No information, however, is available on the quantitative dependence of QO_2 on ATP/ADP ratios in intact cells. Administration of T_3 to euthyroid rats produced a 69% increase in hepatic QO_2 and a 38% fall in the ATP/ADP ratio (Tables I and II). Whether the decline in the ATP/ADP ratio is sufficient to account for the rise in QO_2 cannot be evaluated as yet, for the reasons alluded to above (*i.e.*, heterogeneity in intracellular nucleotide pools and lack of quantitative information on regulation of QO_2 in intact cells). In T_3-treated thyroidectomized rats, there was an insignificant fall in hepatic ATP/ADP ratio in contrast to a 119% increase in QO_2. This finding is consistent with the possibility of simultaneous activation of mitochondrial respiration coupled to phosphorylation and of active Na^+ transport; in effect a combination of mechanisms (a) and (b). Thus, the question of simultaneous activation by parallel pathways of mitochondrial formation of ATP and of Na^+ transport by thyroid hormones remains a distinct possibility.

Summary. The effects of administration of triiodothyronine (T_3) to euthyroid and thyroidectomized rats on hepatic adenine nucleotides were measured using a rapid-freeze technique *in situ*. The respiratory effects (QO_2) of T_3 were measured in parallel groups of rats. Total adenine nucleotides (ATP + ADP + AMP) was unchanged after injections of T_3 in thyroidectomized or euthyroid rats. Administration of T_3 to thyroidectomized rats produced a 119% in-

crease in QO_2 but had no statistically significant effect on ATP or ADP content, or on the ATP/ADP ratio—although the mean value of this ratio was slightly lower after T_3. In euthyroid rats, T_3 stimulated QO_2 by 69% and significantly lowered ATP and raised ADP content; resulting in a 38% decrease in the ATP/ADP ratio. Previous studies implicated an increase in energy utilization by the Na^+ pump in thyroid thermogenesis. It appears unlikely that a primary action of the hormone on mitochondrial metabolism could account for the increase in energy expenditure for Na^+ transport. Combined effects of thyroid hormone via parallel pathways on both mitochondrial oxidative phosphorylation and on the Na^+ pump, however, remains a possibility.

The authors are grateful for the able technical assistance provided by Miss Allison Meader.

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Received June 15, 1973. P.S.E.B.M., 1973, Vol. 144.