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Time-Course of the Effects of Thyroid Hormone on Respiration and $Na^+ + K^+$-ATPase Activity in Rat Liver[1] (38232)

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(Introduced by G. S. Gordan)

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We proposed previously that increased energy utilization for transmembrane active Na^+ transport mediates a significant fraction of the thermogenic action of thyroid hormone (1, 2). In liver, 90% or more of the increase in respiration (Q_{O_2}) produced by injection of triiodothyronine (T_3) into either surgically thyroidectomized or euthyroid rats was attributable to increased energy expenditure in active Na^+ transport. The Na^+ pump-dependent respiration [$Q_{O_2}(t)$] was estimated either by addition of ouabain to or removal of Na^+ from the medium. In addition, we found that a significant thermogenic effect of T_3 was associated with a significant increase in $Na^+ + K^+$-activated adenosinetriphosphatase (NaK-ATPase) activity in liver and kidney homogenates (2). The T_3-induced increase in NaK-ATPase activity was preserved in isolated liver membrane fractions and was differential in that no increase in Mg^{2+}-ATPase or 5′-nucleotidase activity was observed. Valcana and Timiras (3) reported previously that thyroid hormone increased NaK-ATPase activity in fetal rat brain.

The respiratory and enzyme activity measurements described above were made on tissues from rats given repeated doses of T_3 to achieve 'steady state' levels. The present time-course studies were undertaken to answer the following questions: (a) What are the temporal and quantitative relationships during transient and steady-state changes in Q_{O_2} and $Q_{O_2}(t)$ in response to T_3? (b) Do the changes in NaK-ATPase activity vary in proportion to the changes in $Q_{O_2}(t)$ as a function of time?

Methods. Male, Sprague–Dawley rats were used 3–4 wk after surgical thyroidectomy (hypothyroid) or were unoperated (euthyroid). Groups of rats were injected with diluent or Na-L-3,5,3′-triiodothyronine (T_3) 50 μg/100 g body wt, according to the schedules indicated by the arrows on the figures. The rats were decapitated and the livers were transferred to iced, oxygenated Na^+-Ringer's solutions as described previously (1). Slices, 290 μm thick, were prepared immediately with a chopper (Mickle Laboratory Engineering Co., Surrey, England) and transferred to a Warburg respirometer (Aminco, Silver Springs, Md.). Q_{O_2} was measured at 15–30 min intervals at 37°. $Q_{O_2}(t)$ was estimated from Q_{O_2} with and without ouabain (10^{-3} M) in the medium as described previously (1, 2). NaK-ATPase and Mg-ATPase assays were determined on crude homogenates of minced liver (2). Ouabain (10^{-3} M) was used in parallel incubates to estimate $Na^+ + K^+$-

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dependence and the remainder was taken as Mg-ATPase activity (4). The reaction was terminated after 5.0 min by addition of cold trichloroacetic acid (final concentration = 5% by wt). Inorganic phosphate was determined by the method of Fiske and Subbarow (5, 6) and protein by the method of Lowry *et al.* (7). Statistical significance was estimated by the unpaired Student "*t*" test.

Results. 1. *Respiratory Indices.* The effectiveness of the surgical thyroidectomies is clearly indicated by the significantly depressed Q_{O_2} (see the zero-time Q_{O_2}'s of the thyroidectomized (A) and euthyroid rats (B) in Figs. 1 and 2).

The time course of the effects of a single injection of T_3 in hypothyroid rats on Q_{O_2}, $Q_{O_2}(t)$ and the ouabain-insensitive, residual respiration ($Q_{O'_2}$) are shown in Fig. 1A. The zero-time values were determined within one hr after injection of T_3. Total Q_{O_2} increased to a maximum value at 48 hr after injection and declined linearly towards the initial value by day 6. $Q_{O_2}(t)$ rose, peaked at 48 hr and fell thereafter: these changes remained in phase with the changes in Q_{O_2} but the $Q_{O_2}(t)$ did not quite achieve a return to the control value by day 6. Transport-independent respiration, $Q_{O'_2}$, also increased in phase with Q_{O_2} and $Q_{O_2}(t)$ and decreased to the control value by day 6. At the peak of the response (48 hr after injection), the change in $Q_{O_2}(t)$ accounted for 62% and in $Q_{O'_2}$ for 38% of the change in Q_{O_2}. By day 6, $Q_{O_2}(t)$ accounted for all of the 'residual' increase in Q_{O_2} above the control (day 0) level.

The respiratory effects of a single injection of T_3 in euthyroid rats are shown in Fig. 1B. Hepatic Q_{O_2} increased to a peak value at 48 hr and then regressed to about the control level by the 5th day. The time-course of the changes in Q_{O_2} and $Q_{O_2}(t)$ were almost the same. $Q_{O'_2}$ also tended to increase at 24–48 hr but these changes were not statistically significant. At 48 hr, the increase in $Q_{O_2}(t)$ accounted for 65% and in $Q_{O'_2}$ for 35% of the increase in Q_{O_2}. By day 6, Q_{O_2}, $Q_{O_2}(t)$ and $Q_{O'_2}$ well all at control levels.

The results of repeated injections of T_3 (given on days 0, 2 and 4) in hypothyroid rats are shown in Fig. 2A. Q_{O_2} increased to the steady-state level between 2 and 3 days

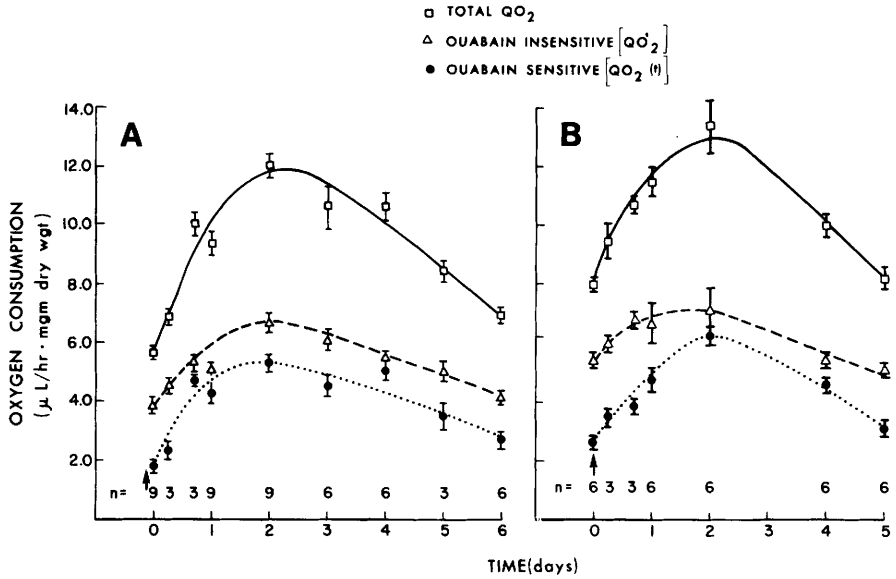

FIG. 1. Time-course of the effects of a single injection of T_3 on oxygen consumption of rat liver. Rats were injected with 50 μg of T_3/100 g body wt on day zero: Panel (A) shows results in thyroidectomized, and Panel (B) in euthyroid rats. The number of rats analyzed at each sampling time is denoted by '*n*'. Each point and vertical line indicates mean ± 1 SEM.

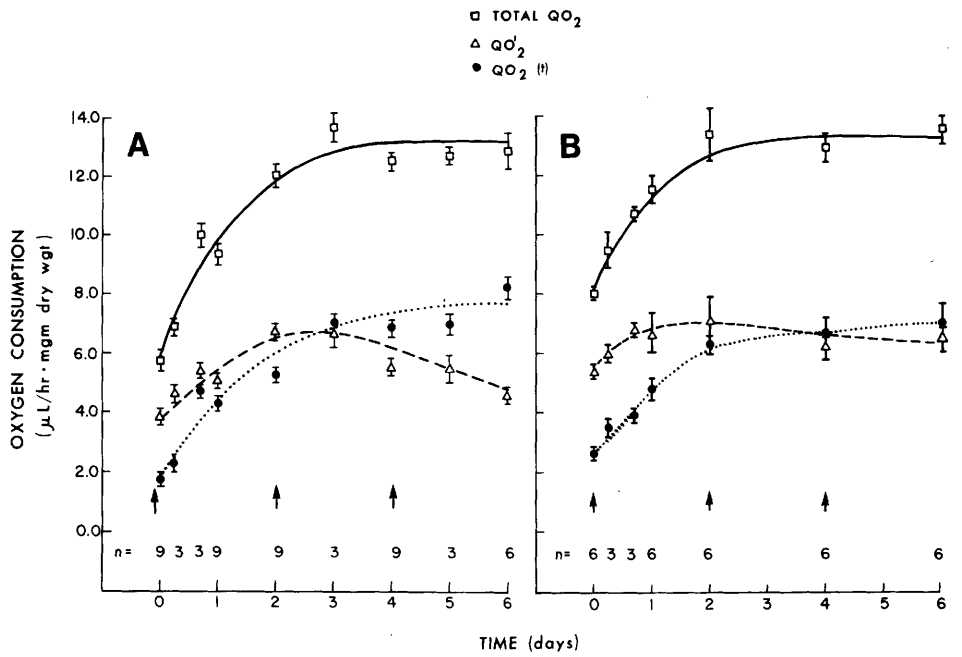

FIG. 2. Time-course of the effects of multiple injections of T_3 on oxygen consumption of rat liver. Rats were injected with 50 μg of T_3/100 g body wt on days 0, 2 and 4 as indicated by the arrows (↑): Panel (A) shows results in thyroidectomized and Panel (B) in euthyroid rats. Steady-state Q_{O_2}s attained hyperthyroid levels in both populations. '*n*' denotes number of rats analyzed at each sampling time. Mean ± 1 SEM.

after the first injection. $Q_{O'_2}$, however, peaked at day 2 and then fell to the baseline level despite continued administration of T_3. In contrast, $Q_{O_2}(t)$ increased continuously (curvilinearly) throughout the 6 days of observation, although most of the effect was achieved by day 3. As reported previously (1), the increment in $Q_{O_2}(t)$ represented ~90% of the increment in Q_{O_2} by the 6th day.

Figure 2B depicts the effects of consecutive injections of T_3 on days 0, 2 and 4 in euthyroid rats. The time-course of the changes in Q_{O_2}, $Q_{O'_2}$ and $Q_{O_2}(t)$ are in distinguishable from those seen in hypothyroid rats (cf. Figs. 2A and 2B). The changes in Q_{O_2} and $Q_{O_2}(t)$ were sustained, whereas the change in $Q_{O'_2}$ was small and not statistically significant. By the 6th day, the change in $Q_{O_2}(t)$ accounted for 80% of the change in Q_{O_2}.

In contrast to the results obtained on injection of T_3, administration of the diluents to both hypothyroid and euthyroid control rats on day 0, 1, 2 and 4 had no effect on hepatic Q_{O_2}, $Q_{O'_2}$ or $Q_{O_2}(t)$. In fact these respiratory indices were the same as the "zero-time" values shown in Figs. 1 and 2.

2. *NaK-ATPase and Mg-ATPase Activities.* In thyroidectomized rats, only steady-state experiments were completed. As shown in Fig. 3A, administration of T_3 (3 doses at 48 hr intervals) resulted in a curvilinear and statistically significant increase in NaK-ATPase activity of crude liver homogenates. The maximum steady state level was attained in 48 hr and was increased 47% above control levels (day 0). Mg-ATPase activity increased 18% (statistically significant) at 48 hr and regressed almost to the baseline value by the 6th day despite continued administration of T_3. A comparison of the enzyme and respiratory changes (cf. Figs. 2A and 3A) indicates that $Q_{O_2}(t)$ and NaK-ATPase varied in parallel as did $Q_{O'_2}$ and Mg-ATPase activity.

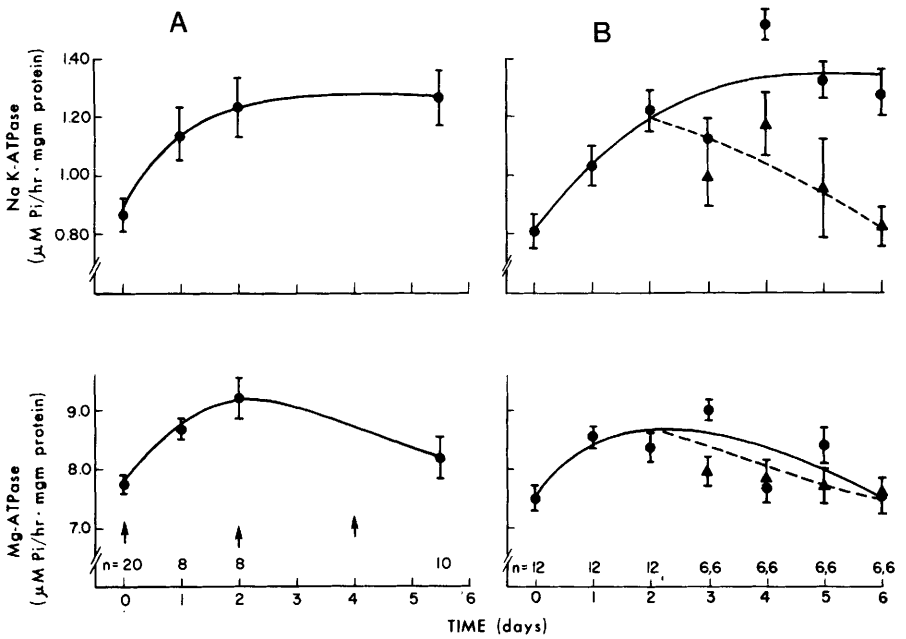

FIG. 3. Time-course of the effects of T_3 on hepatic NaK-ATPase and Mg-ATPase activities. Thyroidectomized rats were injected with 50 μg of T_3/100 g body wt on days 0, 2 and 4 as indicated by the arrows (↑) (Panel (A)). Euthyroid rats were given either a single injection of T_3 on day 0 (- -▲- -) or injected successively on days 0, 2 and 4 (—●—) (Panel (B)). '*n*' denotes the number of rats. Mean ± 1 SEM.

In euthyroid rats, both steady state (3 doses) and treatment state (1 dose) experiments were completed (Fig. 3B). With three consecutive doses of T_3, NaK-ATPase activity rose and reached a plateau that was ~60% above the control level somewhere between the 2nd and 5th days; the scatter in values obtained on the 3rd and 4th day precludes identifying precisely the time at which the maximum was achieved. Nevertheless, there is a definite parallelism in the time-course of the changes in $Q_{O_2}(t)$ and NaK-ATPase activity (cf. Figs. 2B and 3B). With a single injection of T_3, NaK-ATPase activity increased to a peak value at 48 hr (+50%) and then declined to the control level by the 6th day. Although there is some scatter in these results, the zero day and 6 day values are indistinguishable and both are significantly less than the peak value at 48 hr. The changes in $Q_{O_2}(t)$ and NaK-ATPase activity in the transient case were also in phase (cf. Figs. 1B and 3B). As shown in the lower panel of 3B, Mg-ATPase increased 12% in 24 hr and declined (with some fluctuations) to the control level by the 6th day, regardless of whether only one dose or repeated (3 doses) injections were given. Although small in magnitude, the increases in Mg-ATPase activity at 24, 48 and 36 hrs are statistically significant when compared to either the zero or 6 day values.

Discussion. The time-course of the T_3-induced changes in Q_{O_2} of rat liver are similar to previous findings of Barker and Klitgaard (8) with thyroxine (T_4) except that the response was more prolonged. After a single injection of 6 mg of DL-T_4, hepatic Q_{O_2} increased to a broad peak value at 3–5 days and returned to the control level at about the 8th day. In the present study, there were coordinate changes in hepatic Q_{O_2}, $Q_{O_2}(t)$ and NaK-ATPase activity both in the steady-state, multiple injection and transient-state, single injection, experiments. Thus, the inference that stimulation of the Na^+ pump (as manifested by increased NaK-ATPase activity) mediates the increase in $Q_{O_2}(t)$ and a major part of

the hepatic thermogenic response to T_3 remains tenable.

An unexpected finding in this study was the transient increase in the ouabain-insensitive respiration ($Q_{O'_2}$) in both the single and multiple injection experiments in the thyroidectomized rats. The participation of a Na^+-independent pathway in the thermogenic response to T_3 was also evident in earlier studies on hypothyroid skeletal muscle in which the increase in $Q_{O'_2}$ accounted for 55% of the increase in Q_{O_2} (1). In contrast to liver, however, the contribution of $Q_{O'_2}$ to the increase in Q_{O_2} is sustained in skeletal muscle rather than transient. In recent experiments, the increase in skeletal muscle $Q_{O'_2}$ was 50% of the increase in Q_{O_2} after 7 injections of T_3 given every other day over a 2 week period (Asano, Liberman and Edelman, in preparation). Although Mg-ATPase activity in these crude liver homogenates represents a heterogenous mixture of enzymes, it is interesting to note that the time-course of the changes in $Q_{O'_2}$ and Mg-ATPase activity appeared similar and may be related in some way.

Since it has been shown that thyroid hormone increases RNA and protein synthesis, these may be some of the endergonic pathways that contribute to $Q_{O'_2}$. In a steady-state experiment (after 3 injections of T_3), however, we found no significant effect of inhibition of protein synthesis with cycloheximide on rat liver Q_{O_2} or $Q_{O_2}(t)$ (Ismail-Beigi and Edelman, unpublished observations). Nevertheless, the possibility of transient contributions of energy expenditure in RNA and protein synthesis to hepatic thermogenesis deserves further study.

A comparison of the time-course of the parameters we studied with the increase in oxidative capacity of hepatic mitochondria (9), indicates that the increase in Q_{O_2}, $Q_{O_2}(t)$ and NaK-ATPase activity precede the effect on mitochondrial respiration by at least 12 hr. Babior *et al.* (10) found that thyroid hormone (*in vivo*) increased atractyloside-sensitive ADP uptake by isolated rat liver mitochondria 1 to 3 days after administration of the hormone. Thus, thyroid hormone may stimulate the Na^+ pump and mitochondrial oxidative capacity by independent and parallel pathways. The possibility that enhanced ADP production secondary to activation of the Na^+ pump is involved in the induction of the changes in mitochondrial activity, however, also deserves further consideration.

Summary. As a further test of the hypothesis that increased energy expenditure in active Na^+ transport mediates a major part of thyroid thermogenesis, time-course measurements were made of hepatic oxygen consumption (Q_{O_2}), Na^+-dependent oxygen consumption [$Q_{O_2}(t)$] and $Na^+ + K^+$ adenosine-triphosphatase (NaK-ATPase) activity after a single or repeated injections of triiodothyronine (T_3) into thyroidectomized and euthyroid rats. In both groups, after single injections, Q_{O_2} and $Q_{O_2}(t)$ increased to peak values at 48 hr and declined in parallel to close to the control level by the 6th day. In euthyroid rats, NaK-ATPase activity mainfested the same time-course with a peak increase at 48 hr. In both groups, after repeated injections (every other day $\times 3$), there were curvilinear increases in Q_{O_2}, $Q_{O_2}(t)$ and NaK-ATPase activity to steady-state levels in 2–3 days. These findings are in accord with the inference that thyroidal stimulation of the Na^+ pump mediates the increase in $Q_{O_2}(t)$. In addition, we found that in hypothyroid rats there was an increase in Na^+-independent respiration and Mg-ATPase activity with a peak response at 48 hr; this effect was transient even with repeated injections of T_3.

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