The Mechanism of the Calorigenic Action of Thyroid Hormone

Stimulation of $Na^+ + K^+$-activated adenosinetriphosphatase activity

FARAMARZ ISMAIL-BEIGI and ISIDORE S. EDELMAN

From the Cardiovascular Research Institute and the Departments of Medicine, and Biochemistry and Biophysics, University of California, San Francisco, California 94122

ABSTRACT In an earlier study, we proposed that thyroid hormone stimulation of energy utilization by the Na^+ pump mediates the calorigenic response. In this study, the effects of triiodothyronine (T_3) on total oxygen consumption (Q_{O_2}), the ouabain-sensitive oxygen consumption [$Q_{O_2}(t)$], and NaK-ATPase in liver, kidney, and cerebrum were measured. In liver, ~90% of the increase in Q_{O_2} produced by T_3 in either thyroidectomized or euthyroid rats was attributable to the increase in $Q_{O_2}(t)$. In kidney, the increase in $Q_{O_2}(t)$ accounted for 29% of the increase in Q_{O_2} in thyroidectomized and 46% of the increase in Q_{O_2} in euthyroid rats. There was no demonstrable effect of T_3 in euthyroid rats on Q_{O_2} or $Q_{O_2}(t)$ of cerebral slices. The effects of T_3 on NaK-ATPase activity in homogenates were as follows: In liver +81% from euthyroid rats and +54% from hypothyroid rats. In kidney, +21% from euthyroid rats and +69% from hypothyroid rats. T_3 in euthyroid rats produced no significant changes in NaK-ATPase or Mg-ATPase activity of cerebral homogenates. Liver plasma membrane fractions showed a 69% increase in NaK-ATPase and no significant changes in either Mg-ATPase or 5′-nucleotidase activities after T_3 injection. These results indicate that thyroid hormones stimulate NaK-ATPase activity differentially. This effect may account, at least in part, for the calorigenic effects of these hormones.

INTRODUCTION

The calorigenic action of thyroid hormones [thyroxin (T_4) and triiodothyronine (T_3)] in homoiothermic adult vertebrates is exerted on many tissues (e.g., skeletal and cardiac muscle, liver, kidney; brain is a notable exception) and is preserved in surviving tissue preparations in vitro (1, 2). The available evidence indicates that induction of RNA and protein synthesis mediates both the calorigenic and morphogenetic actions of these hormones

(3). Administration of thyroid hormone produces an increase in the number, size, and oxidative and phosphorylative capacity of mitochondria of mammalian skeletal muscle, and causes an increase in the size of mitochondria as well as proliferation of the endoplasmic reticulum in amphibian liver (4–7). In the steady state, however, a sustained increase in mitochondrial respiration coupled to oxidative phosphorylation requires a sustained increase in ATP utilization.

In an earlier study, we examined the hypothesis that activation of energy utilization by transmembrane active Na^+ transport is one of the primary mediators in the calorigenic response to thyroid hormone (8). In euthyroid rats more than 90% of the increment in Q_{O_2} in liver and skeletal muscle produced by injections of T_3 and T_4 was attributable to increased energy utilization by the Na^+ pump. In T_3-treated thyroidectomized rats, activation of Na^+ transport accounted for about 45% of the respiratory increment in skeletal muscle and more than 90% of the increment in liver. We also found that in euthyroid rats, T_3 reduced intracellular Na^+ and raised intracellular K^+ concentrations in liver and diaphragm. These results suggested that hormonal activation of Na^+ and K^+ transport was predominantly a result of either a local increase in ATP concentration (or ATP:ADP ratios) or direct stimulation of the Na^+ pump. Skou (9) has provided extensive evidence that the membrane-bound $Na^+ + K^+$–activated adenosine-triphosphatase (NaK-ATPase) is an enzymatic expression of the mechanism for active transport of Na^+ and linked Na^+-K^+ transport across cell membranes. Accordingly, to obtain additional information on the mechanisms involved in thyroid calorigenesis, we made comparative measurements of the effects of T_3 on Q_{O_2}, $Q_{O_2}(t)$, and NaK-ATPase and Mg-ATPase activity in liver, kidney, and brain cortex.

METHODS

Male, Sprague-Dawley rats (200–250 g body weight) were maintained on Purina chow ad lib. Thyroidectomy was performed surgically and the animals were used 3–4 wk postoperatively. The euthyroid or thyroidectomized rats received three injections of Na–L-3,5,3′ triiodothyronine (T_3) at a dosage of 50 μg T_3/100 g body weight on alternate days subcutaneously.[1] Paired control rats were injected with a comparable volume of the diluent (5×10^{-4} M NaOH) according to the same schedule. The animals were killed by decapitation and the organs to be studied were transferred to iced, oxygenated, modified Na^+-Ringer solution (mM) [Na^+, 135; K^+, 5.0; Mg^{++}, 0.5; Ca^{++}, 1.0; Cl^-, 139; $H_2PO_4^-$, 5.0; Tris base, 5.0; glucose = 10; pH = 7.40 and osmolality, 290 milliosmols per liter]. Renal papillary tissue was removed by sharp dissec-

[1] This dose of T_3 produces an increase in Q_{O_2} which is less than twofold and is within the range of effects seen in spontaneous hyperthyroidism (8). The peak effect of T_3 with respect to BMR occurs at ~48 hr (10). The sequence of doses was spaced in order to achieve a steady-state increase in Q_{O_2}.

tion before preparing the kidney slices. Tissue slices were prepared immediately with a tissue chopper (The Mickle Laboratory Engineering Co., Surrey, England). Cerebral and hepatic slices were 290 μ and kidney slices 275 μ in thickness. Care was taken so that the time of tissue preparation in the cold was constant at 15 min. The slices were transferred to a Warburg respirometer (Aminco, Silver Springs, Md.) and respiratory rates measured at 15–30 min intervals at 37°C for 2 hr as described previously (11). Ouabain, a specific inhibitor of NaK-ATPase, was used at 10^{-3} M for the determination of $Q_{O_2}(t)$ which was taken as a measure of energy utilization by the Na^+ pump (8, 9, 12). The dry weight of tissue in each flask was determined by removing the slices, blotting briefly with filter paper, and heating at 91°C for 24 hr in tared aluminum cups. The Q_{O_2} data are expressed as microliters of oxygen per hour per milligram dry weight of tissue.

NaK-ATPase and Mg-ATPase activity were determined in tissues from diluent-injected controls or rats treated with T_3 according to the dosage schedule described above. Approximately 1 g of wet weight of tissue was minced and homogenized in 10 ml of solution containing 0.25 M sucrose, 1.25 mM EGTA, and 10 mM Tris, pH 7.0, using six strokes in a Teflon-glass Elvehjem-Potter homogenizer at 2–3°C. The homogenates were passed through gauze and diluted further in the homogenizing medium (1:15 for cerebrum, 1:5 for kidney, 1:3 for liver) so that less than 10% of ATP would be split during the incubation period. The homogenates were warmed to 37°C for 5 min prior to initiation of the reaction. The reaction was started by the addition of 100 μl of homogenate to 900 μl of reaction medium giving final concentrations (all in mM) of ATP, 5.0; Mg^{++}, 5.0; Na^+, 120; K^+, 12.5; Tris, 25; Cl^-, 137.5; azide, 5.0; pH, 7.40, and maintained at 37°C. Azide was included to prevent regeneration of ATP by the mitochondria present in the homogenate. Ouabain (10^{-3} M) was used in each set of incubations to estimate the NaK-ATPase activity, the remainder was taken as Mg-ATPase activity (9). The reaction was terminated after 5.0 min by the addition of cold TCA (final concentration = 5% by weight) to the flasks. The liberated inorganic phosphate content was determined by the Fiske and Subbarow method (13, 14) and protein content by the method of Lowry et al. (15). The protein standards were prepared in 20 mM Tris buffer, precipitated with cold TCA as in the unknowns, and the blank values were corrected accordingly. Fujita et al. (16) reported that in sodium iodide treated–microsomal fractions from pig brain they could demonstrate four apparently different ouabain-sensitive ATPase activities under various experimental conditions. Ouabain at 10^{-4} M inhibited part of the Mg^{++}-ATPase activity only at relatively low pH. However, in assays for ATPase activity in cat liver homogenates (17), calf cardiac microsomal preparations (18), rat liver plasma membrane fractions (19), and rat liver homogenates (20), it has been shown that the ouabain-sensitive ATPase activity is quantitatively equivalent to the reduction in ATPase activity when Na^+ and K^+ are omitted from the reaction mixture. Accordingly, we used susceptibility of ATPase activity to ouabain as an index of NaK-ATPase activity.

In order to obtain additional information on the degree of selectivity in the effects of thyroid hormone on membrane-bound enzymes, comparative Mg-ATPase, NaK-ATPase, and 5′-nucleotidase assays were performed on liver plasma membrane fractions prepared by the modified method of Neville (21, 22). The assays for Mg-ATPase

and NaK-ATPase activity were as described above except that azide was not added to the reaction mixtures. The 5′-nucleotidase activity was determined in the same medium as in the assays for total ATPase activity, except for the substitution of 5′-AMP (final concentration, 5.0 mM) for ATP (23).

All results were calculated as the mean ± SE of the mean. The "p" values were obtained from the Student t test (24).

RESULTS

In order to provide a direct comparison of the respiratory effects of thyroid hormone and the dependence of these effects on Na^+ transport among liver, kidney, and brain, we repeated the studies on liver that were reported previously (8).

In liver slices from euthyroid rats, ouabain reduced the average Q_{O_2} by about 38%; Na^+ transport-dependent respiration averaged 3.1 ± 0.2 μl/hr/mg dry weight (Table I). Administration of T_3 increased Q_{O_2} by ~50% and

TABLE I

EFFECT OF OUABAIN ON Q_{O_2} OF LIVER SLICES FROM EUTHYROID RATS (±T_3)*

Ouabain	Euthyroid	Euthyroid + T_3	Δ	p
0	8.2 ± 0.3	12.4 ± 0.4	4.2	<0.001
10^{-3} M	5.1 ± 0.2	5.5 ± 0.3	0.4	n.s.‡
$Q_{O_2}(t)$:	3.1 ± 0.2	6.9 ± 0.3	3.8	<0.001

* Q_{O_2} expressed as microliters oxygen per hour per milligram dry weight. Mean ± SEM. (n = 14).
$Q_{O_2}(t)$ denotes the ouabain-sensitive Q_{O_2}.
‡ n.s. denotes p value of >0.05.

$Q_{O_2}(t)$ by more than 120%. Inhibition of Na^+ transport activity virtually eliminated the respiratory effects of T_3. These results are in close accord with those reported previously (8) and imply that >90% of the increase in the Q_{O_2} in the transition from the euthyroid to the hyperthyroid state in liver is attributable to an increase in energy utilization by the Na^+ pump. Similarly, as shown in Table II, administration of T_3 to hypothyroid rats produced a striking increase in Q_{O_2}, almost doubling the base line value, but had no effect on the respiratory rates of treated and control slices in the presence of 10^{-3}M ouabain (i.e., 4.0 ± 0.3 vs. 3.9 ± 0.4). Thus, all the T_3-produced increment in Q_{O_2} is accounted for in the increase in $Q_{O_2}(t)$ from 1.7 ± 0.2 to 7.2 ± 0.6. These results are also in close agreement with our earlier studies (8).

In the euthyroid kidney slices, 36% of the Q_{O_2} was ouabain-inhibitable (Table III). Similar results have been reported in rabbit kidney cortex (11).

Injections of T_3 produced a 22% increase in Q_{O_2} and a concomitant 28% increase in $Q_{O_2}(t)$. The increase in $Q_{O_2}(t)$ accounted for 46% of the T_3-dependent increment in Q_{O_2}, which implies that slightly more than half of the increase in respiration is independent of the effects of T_3 on ouabain-sensitive Na^+ transport systems. In the kidney slices from hypothyroid rats

TABLE II

EFFECT OF OUABAIN ON Q_{O_2} OF LIVER SLICES FROM THYROIDECTOMIZED RATS ($\pm T_3$)*

Ouabain		Hypothyroid	Hypothyroid + T_3	Δ	p
0		5.7 ± 0.3	11.1 ± 0.7	5.4	<0.001
10^{-3} M		4.0 ± 0.3	3.9 ± 0.4	−0.1	n.s.
	$Q_{O_2}(t)$:	1.7 ± 0.2	7.2 ± 0.6	5.6	<0.001

* Q_{O_2} expressed as microliters oxygen per hour per milligram dry weight. Mean ± SEM. (n = 10).

TABLE III

EFFECT OF OUABAIN ON Q_{O_2} OF KIDNEY SLICES FROM EUTHYROID RATS ($\pm T_3$)*

Ouabain		Euthyroid	Euthyroid + T_3	Δ	p
0		26.2 ± 0.4	31.9 ± 0.6	5.7	<0.001
10^{-3} M		16.9 ± 0.4	20.0 ± 0.4	3.1	<0.001
	$Q_{O_2}(t)$:	9.3 ± 0.5	11.9 ± 0.5	2.6	<0.005

* Q_{O_2} expressed as microliters oxygen per hour per milligram dry weight. Mean ± SEM. (n = 13).

TABLE IV

EFFECT OF OUABAIN ON Q_{O_2} OF KIDNEY SLICES FROM THYROIDECTOMIZED RATS ($\pm T_3$)*

Ouabain		Hypothyroid	Hypothyroid + T_3	Δ	p
0		18.6 ± 0.4	28.6 ± 0.5	10.0	<0.001
10^{-3} M		12.8 ± 0.4	19.9 ± 0.3	7.1	<0.001
	$Q_{O_2}(t)$:	5.8 ± 0.2	8.7 ± 0.4	2.9	<0.001

* Q_{O_2} expressed as microliters oxygen per hour per milligram dry weight. Mean ± SEM. (n = 12).

injection of T_3 resulted in a 54% increase in Q_{O_2} and a 50% increase in $Q_{O_2}(t)$ (Table IV). The increase in $Q_{O_2}(t)$ accounted for 29% of the T_3-produced increment in Q_{O_2}, which implies that ~70% of this effect was not dependent on the ouabain-inhibitable component of Na^+ transport.

The effect of injections of T_3 on the respiration of cerebral slices is given in Table V. In euthyroid cerebral slices ~40% of the Q_{O_2} was inhibited by

ouabain which is in accord with the findings of Whittam (25). There was no detectable change in either total Q_{O_2} or $Q_{O_2}(t)$ after T_3 injection. These findings agree with earlier studies in which adult brain did not respond calorigenically to thyroid hormone (1, 2).

The results summarized in Tables I–V suggest that virtually all the calorigenic response to thyroid hormone in the liver is a result of activation of the Na^+ pump, that a similar mechanism accounts for one-third to one-half of the effect in the kidney, and that in euthyroid rats, the brain shows neither a calorigenic nor a Na^+ pump effect.

TABLE V

EFFECT OF OUABAIN ON Q_{O_2} OF CEREBRAL SLICES FROM EUTHYROID RATS ($\pm T_3$)*

Ouabain	Euthyroid	Euthyroid + T_3	Δ	p
0	10.3 ± 0.2	10.2 ± 0.3	−0.1	n.s.
10^{-3} M	6.2 ± 0.2	6.6 ± 0.2	0.4	n.s.
$Q_{O_2}(t)$:	4.1 ± 0.2	3.6 ± 0.3	−0.5	n.s.

* Q_{O_2} expressed as microliters oxygen per hour per milligram dry weight. Mean ± SEM. (n = 10).

TABLE VI

ATPase ACTIVITY OF LIVER HOMOGENATES FROM EUTHYROID RATS ($\pm T_3$)*

	Mg-ATPase	NaK-ATPase
	μmoles Pi/hr per mg protein	
Euthyroid	6.06 ± 0.28	0.62 ± 0.05
Euthyroid + T_3	6.94 ± 0.43	1.12 ± 0.05
Δ	0.88	0.50
p	n.s.	<0.001

* Mean ± SEM. (n = 11).

As an approach to the mechanism of thyroid hormone stimulation of energy utilization by the Na^+ transport process, we measured NaK-ATPase activity in crude homogenates from liver, kidney, and brain. The effects of injections of T_3 in euthyroid rats on Mg-ATPase and NaK-ATPase activity in crude liver homogenates are summarized in Table VI. The transition from the euthyroid to the hyperthyroid state produced an 81% increase in NaK-ATPase activity (highly significant) and only a 15% increase in the Mg-ATPase activity, which was not statistically significant. As shown in Table VII, injection of T_3 in hypothyroid rats resulted in a 54% increase in NaK-ATPase activity compared to a 27% increase in Mg-ATPase activity in crude liver homogenates. Although direct comparisons of hypothyroid and

euthyroid enzyme profiles are complicated by the possibility of many secondary effects of the prolonged athyroid state, it is noteworthy that the absolute specific activities of the NaK-ATPase were somewhat higher in the control hypothyroid compared to the euthyroid liver homogenates (i.e., 0.82 ± 0.11 vs. 0.62 ± 0.05 μmole Pi/hr/mg protein).

The effects of T_3 in euthyroid and hypothyroid rats on enzyme activities in renal homogenates are summarized in Tables VIII and IX. Treatment

TABLE VII

ATPase ACTIVITY OF LIVER HOMOGENATES FROM THYROIDECTOMIZED RATS (±T_3)*

	Mg-ATPase	NaK-ATPase
	μmoles Pi/hr per mg protein	
Hypothyroid	6.53 ± 0.23	0.82 ± 0.11
Hypothyroid + T_3	8.26 ± 0.41	1.26 ± 0.11
Δ	1.73	0.44
p	<0.010	<0.025

* Mean ± SEM. (n = 10).

TABLE VIII

ATPase ACTIVITY OF KIDNEY HOMOGENATES FROM EUTHYROID RATS (±T_3)*

	Mg-ATPase	NaK-ATPase
	μmoles Pi/hr per mg protein	
Euthyroid	16.8 ± 0.8	10.0 ± 0.5
Euthyroid + T_3	18.6 ± 0.5	12.1 ± 0.7
Δ	1.8	2.1
p	n.s.	<0.05

* Mean ± SEM. (n = 11).

with T_3 resulted in a 21% increase in NaK-ATPase activity and an insignificant change in Mg-ATPase activity in the euthyroid case. More pronounced effects were seen in the hypothyroid animals in which T_3 produced a 69% increase in NaK-ATPase activity and had no appreciable effect on Mg-ATPase activity.

In contrast to the findings in liver and kidney, administration of T_3 to euthyroid rats caused no demonstrable difference in Mg-ATPase or NaK-ATPase activities of the cerebral homogenates (Table X).

The observed changes in NaK-ATPase activity might bear a quantitative relationship to the changes in Na^+ transport-dependent respiration, $Q_{O_2}(t)$, if there is proportionality between the activity of the Na^+ pump in vivo

and the activity of the enzyme assayed in vitro. A comparison of the average effects of T_3 on $Q_{O_2}(t)$ and NaK-ATPase activity in brain, kidney, and liver is shown in Fig. 1. These data were computed from the absolute values given in Tables I–X. In brain, neither $Q_{O_2}(t)$ nor NaK-ATPase activity was significantly altered by injections of T_3 in euthyroid rats. In kidney, the changes in $Q_{O_2}(t)$ and NaK-ATPase activity were approximately proportional after administration of T_3 to euthyroid and hypothyroid animals. In liver from euthyroid rats, T_3 produced a somewhat greater rise in $Q_{O_2}(t)$ than in NaK-

TABLE IX

ATPase ACTIVITY OF KIDNEY HOMOGENATES FROM THYROIDECTOMIZED RATS ($\pm T_3$)*

	Mg-ATPase	NaK-ATPase
	μmoles Pi/hr per mg protein	
Hypothyroid	18.0 ± 1.2	7.3 ± 0.6
Hypothyroid + T_3	19.7 ± 1.2	12.3 ± 0.6
Δ	1.7	5.0
p	n.s.	<0.001

* Mean ± SEM. (n = 11).

TABLE X

ATPase ACTIVITY OF CEREBRAL HOMOGENATES FROM EUTHYROID RATS ($\pm T_3$)*

	Mg-ATPase	NaK-ATPase
	μmoles Pi/hr per mg protein	
Euthyroid	14.1 ± 0.5	16.9 ± 0.6
Euthyroid + T_3	15.2 ± 0.5	15.9 ± 0.4
Δ	1.1	−1.0
p	n.s.	n.s.

* Mean ± SEM. (n = 12).

ATPase activity but the magnitude of these effects was not too dissimilar. In liver from hypothyroid rats, T_3 had a far greater effect on $Q_{O_2}(t)$ than on NaK-ATPase activity, although the enzyme response was statistically significant. Despite this discrepancy, however, a significant calorigenic effect was in all instances associated with a significant increase in NaK-ATPase activity in the homogenates. It is pertinent, therefore, to inquire into the possibility that the effect of the thyroid hormone on NaK-ATPase activity is preserved in partially purified cell membrane fractions and to ask whether the effect is specific or is shared by other membrane-bound enzymes.

In order to make these comparisons, we chose membrane-bound Mg-

ATPase and 5′-nucleotidase activities (23). The liver membrane fractions were prepared by the method of Neville (21) as modified by Emmelot and Bos (22). The results are given in Table XI and indicate that preparation of the membrane fraction compared to the crude homogenates resulted in

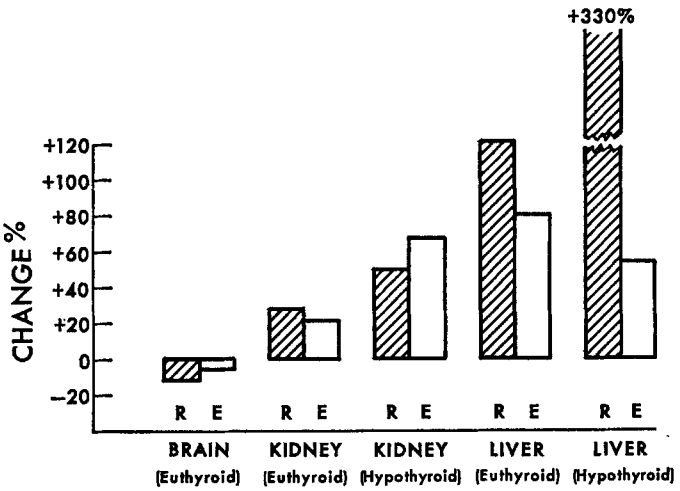

FIGURE 1. The effects of administration of T_3 in vivo on Na^+ transport-dependent respiration and NaK-ATPase activity assayed in vitro. R denotes $Q_{O_2}(t)$ of tissue slices defined by sensitivity to ouabain (10^{-3} M) and E denotes NaK-ATPase activity in crude homogenates. The designations euthyroid and hypothyroid refer to the state of the animal at the time when T_3 injections were started. Per cent change represents the differences seen between the T_3-injected and diluent-injected control populations. The height of the bars represents average values computed from Tables I–X. The per cent changes in $Q_{O_2}(t)$ and NaK-ATPase activity in kidney and liver were all significant at the 5% level of confidence.

TABLE XI

ENZYME ACTIVITIES OF LIVER PLASMA MEMBRANE FRACTION FROM EUTHYROID RATS ($\pm T_3$)*

	Mg-ATPase	NaK-ATPase	5′ Nucleotidase
Euthyroid	147 ± 11	11.3 ± 0.8	179 ± 25
Euthyroid + T_3	148 ± 7	19.1 ± 1.4	139 ± 11
Δ	1	7.8	−40
p	n.s.	<0.001	n.s.

* Enzyme activities are expressed as micromoles Pi released per hour per milligram protein. Mean ± SEM. (n = 20 for Mg-ATPase and NaK-ATPase, n = 10 for 5′ nucleotidase).

about a 20-fold increase in the specific activities of Mg-ATPase and NaK-ATPase (cf. Tables VI and XI). Injection of euthyroid rats with T_3 elicited a 69% increase in the specific activity of NaK-ATPase (i.e., from 11.3 to 19.1 μmoles Pi/hr/mg protein). In contrast, the activity of Mg-ATPase

was unchanged and 5′-nucleotidase activity was less in the T_3-treated group, although this difference was not statistically significant. Thus, the thyroid-stimulated increase in NaK-ATPase activity was preserved in the isolation of the plasma membranes, and the effect appears to be selective inasmuch as no increase in the activity of Mg-ATPase or 5′-nucleotidase was observed.

DISCUSSION

In the rat, the calorigenic action of thyroid hormone is characterized by a latent period of ~12 hr and a peak response in ~48 hr after a single injection (1, 10). The respiratory effect is preserved in surviving skeletal or cardiac muscle, liver, and kidney in vitro (1, 2). Furthermore, this effect requires intact mechanisms for RNA and protein synthesis (3, 10).

In the present study, we used sensitivity to ouabain as the index of the fraction of total respiration that was dependent on active Na^+ transport based on the evidence that ouabain is a specific inhibitor of the Na^+ pump and of the transport enzyme, NaK-ATPase (8, 9, 12). The specificity of ouabain, at a concentration of 10^{-3} M, as an inhibitor of the Na^+ pump was previously tested in rat liver and diaphragm; no effect on Q_{O_2} was observed in Na^+-free media in these tissues (8). It is possible, however, that changes in cell composition (e.g., K^+ concentration) during incubation in ouabain may influence the magnitude of $Q_{O_2}(t)$ and the over-all effect. This possibility deserves further study.

The present results confirm our earlier findings of the close correlation between the T_3-dependent increase in Q_{O_2} and $Q_{O_2}(t)$ in liver. In addition, in the kidney 29 and 46% of the increases in Q_{O_2} were attributable to the increases in $Q_{O_2}(t)$ after administration of T_3 to hypothyroid and euthyroid rats, respectively. It is possible that a significant part of the ouabain-insensitive increase in Q_{O_2} of the kidney produced by T_3 could be a consequence of an associated increase in ouabain-insensitive ion transport. Kleinzeller and Knotková (26) reported that 0.3 mM ouabain reduced the ^{24}Na efflux rate constant by only 50% in rabbit kidney cortex slices. Their results imply the existence of a significant ouabain-independent Na^+ transport system in the kidney. An additional possibility is that a considerable fraction of the increase in ouabain-insensitive Q_{O_2} is involved in the energy requirements for morphogenesis. We found an approximate doubling in the mass of the kidney during the 6 days of treatment with T_3 in hypothyroid rats. Administration of T_3 to euthyroid rats had no appreciable effect on either Q_{O_2} or $Q_{O_2}(t)$ in brain cortex.

With the exception of the response to T_3 of the liver in hypothyroid rats, there was a reasonable correspondence between the increase in $Q_{O_2}(t)$ and in NaK-ATPase activity measured under V_{max} conditions (Fig. 1). The differential increase in the activities of NaK-ATPase compared to those of Mg-ATPase and 5′-nucleotidase may reflect thyroid hormone induction of the

synthesis of NaK-ATPase, thereby increasing the number of pump sites per unit mass of the plasma membrane. Alternatively, this differential increase could be a result of the presence of an activator of NaK-ATPase activity which was retained in the membrane fraction.

The studies of Kawada et al. (27) and of Valcana and Timiras (28) also indicate effects of thyroid hormone on NaK-ATPase activity. Metamorphosis in frogs, which is a thyroid hormone–dependent process, resulted in a threefold increase in NaK-ATPase of the epidermis (27). Although the adult mammalian brain does not respond calorigenically to thyroid hormone, the developing brain responds both calorigenically and morphogenetically during maturation. Extirpation of the thyroid gland in immature rats retarded the rise in brain NaK-ATPase activity, and the accumulation of K^+, and extrusion of Na^+ and Cl^- seen during normal maturation (28).

Adenyl cyclase is another membrane-bound enzyme system that may be involved in thyroid calorigenesis. Krishna et al. (29) found that injection of thyroid hormones in rats elicited an increase in adenyl cyclase activity in fat pads and the effect depended on intact RNA and protein synthesis. Levey and Epstein (30) observed activation of myocardial adenyl cyclase by direct addition of T_3 and T_4 to the incubating medium. The relevance of direct activation of adenyl cyclase in vitro to thyroid calorigenesis, however, is obscure as the latent period of $\sim$12 hr seen in vivo and the lack of an effect of direct addition of thyroid hormones to intact tissues in vitro argue for an indirect rather than a local activating effect (31).

The direction of change in intracellular Na^+ and K^+ concentrations is in accord with the inference of thyroid hormone stimulation of the Na^+ pump (8). The ionic changes, however, were modest compared to the magnitude of change in either $Q_{O_2}(t)$ or NaK-ATPase activity. In the intact cell, the respiratory response may be a consequence of simultaneous effects on the permeability of the plasma membrane to Na^+ and K^+, activation of NaK-ATPase activity, and a shift in the sensitivity of the mitochondrial system to ATP/ADP levels. That the active transport and passive permeability pathways may be intimately related was revealed in the studies on low and high K^+ sheep erythrocytes by Tosteson and Hoffman (32). If thyroid hormone–induced calorigenesis is mediated by simultaneous effects on active and passive transport, the direction of change in cellular Na^+ and K^+ concentrations indicates that the dominant effect is on the Na^+ pump.

The disproportionate increase in $Q_{O_2}(t)$ compared to NaK-ATPase activity in liver after T_3 administration to hypothyroid rats raises the question of the determinants of the quantitative relationship between ouabain-sensitive respiration and enzymic effects. It is possible, if not probable, that in vivo the Na^+ pump operates below the V_{max} level. Since our assays on NaK-ATPase

activity were carried out under V_{max} conditions, the disproportionate effect on $Q_{O_2}(t)$ could be a result of changes in the K_m of the Na^+ pump. Thus, further studies are needed on the effects of thyroid hormones on NaK-ATPase kinetics.

We are indebted to Allison Meader for her able technical assistance.

This work is part of the dissertation to be submitted by F. Ismail-Beigi in partial fulfillment of the requirements for a Ph.D. degree in Biophysics at the University of California at San Francisco.

Dr. Ismail-Beigi is the recipient of an Advanced Research Fellowship of the Bay Area Heart Association.

This study was supported in part by Program Project Grant HE-06285 from the National Heart and Lung Institute, National Institutes of Health.

Received for publication 9 December 1970.

REFERENCES

1. BARKER, S. B., and H. M. KLITGAARD. 1952. Metabolism of tissues excised from thyroxin-injected rats. *Amer. J. Physiol.* **170**:81.
2. BARKER, S. B. 1964. Physiological activity of thyroid hormones and analogues. *In* The Thyroid Gland. R. Pitt-Rivers and W. R. Trotter, editors. Butterworth and Co. (Publishers) Ltd., London. **1**:199.
3. TATA, J. R. 1968. Co-ordinated formation of membranes and biosynthetic activity during growth and development. *In* Regulatory Functions of Biological Membranes. J. Järnefelt, editor. Biochimica and Biophysica Acta Library. Series **11**:222. Elsevier Publishing Co., Amsterdam.
4. TATA, J. R., L. ERNSTER, O. LINDBERG, E. ARRHENIUS, S. PEDERSON, and R. HEDMAN. 1963. The action of thyroid hormones at the cell level. *Biochem. J.* **86**:408.
5. LEE, Y., and H. A. LARDY. 1965. Influence of thyroid hormone on L-α-glycerophosphate dehydrogenase and other dehydrogenases in various organs of the rat. *J. Biol. Chem.* **240**:1427.
6. GUSTAFSSON, R., J. R. TATA, O. LINDBERG, AND L. ERNSTER. 1965. Relationship between structure and activity of rat skeletal muscle mitochondria after thyroidectomy and thyroid hormone treatment. *J. Cell Biol.* **26**:555.
7. BENNETT, T. P., J. S. GLENN, and H. SHELDON. 1970. Changes in the fine structure of tadpole (Rana catesbeiana) liver during thyroxin-induced metamorphosis. *Develop. Biol.* **22**:232.
8. ISMAIL-BEIGI, F., and I. S. EDELMAN. 1970. The mechanism of thyroid calorigenesis: Role of active sodium transport. *Proc. Nat. Acad. Sci. U.S.A.* **67**:1071.
9. SKOU, J. C. 1965. Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. *Physiol. Rev.* **45**:596.
10. TATA, J. R. 1963. Inhibition of the biological action of thyroid hormones by actinomycin D and puromycin. *Nature* (*London*). **197**:1167.
11. UMBREIT, W. W., R. H. BURRIS, and J. F. STAUFFER. 1957. Manometric Techniques. Burgess Publishing Co., Minneapolis.
12. WHITTAM, R., and J. S. WILLIS. 1963. Ion movements and oxygen consumption in kidney cortex slices. *J. Physiol.* (*London*). **168**:158.
13. FISKE, C. R., and Y. SUBBAROW. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**:375.
14. LOWRY, O. H., and J. A. LOPEZ. 1946. The determination of inorganic phosphate in the presence of labile phosphate esters. *J. Biol. Chem.* **162**:421.
15. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.
16. FUJITA, M., K. NAGANO, N. MIZUNO, Y. TASHIMA, T. NAKAO, and M. NAKAO. 1968. Com-

parison of some minor activities accompanying a preparation of sodium-plus-potassium ion–stimulated adenosine triphosphatase from pig brain. *Biochem. J.* **106**:113.

17. BONTING, S. L., K. A. SIMON, and N. M. HAWKINS. 1961. Studies on sodium-potassium–activated adenosine triphosphatase. I. Quantitative distribution in several tissues of the cat. *Arch. Biochem. Biophys.* **95**:416.
18. MATSUI, H., and S. A. SCHWARTZ. 1966. Purification and properties of a highly active ouabain-sensitive Na^+,K^+-dependent adenosine triphosphatase from cardiac tissue. *Biochim. Biophys. Acta.* **128**:380.
19. EMMELOT, P., and C. J. BOS. 1966. Studies on plasma membranes. III. Mg^{++}-ATPase, $(Na^+$-K^+-$Mg^{++})$–ATPase and 5′-nucleotidase activity of plasma membranes isolated from rat liver. *Biochim. Biophys. Acta.* **120**:369.
20. BAKKEREN, J. A. J. M., and S. L. BONTING. 1968. Studies on $(Na^+$-$K^+)$–activated ATPase. XX. Properties of $(Na^+$-$K^+)$–activated ATPase in rat liver. *Biochim. Biophys. Acta.* **150**:460.
21. NEVILLE, D. M., JR. 1960. The isolation of a cell membrane fraction from rat liver. *J. Biophys. Biochem. Cytol.* **8**:413.
22. EMMELOT, P., and C. J. BOS. 1962. Adenosine triphosphatase in cell-membrane fraction from rat liver. *Biochim. Biophys. Acta.* **58**:374.
23. EMMELOT, P., C. J. BOS, E. L. BENEDETTI, and P. RUMKE. 1964. Studies on plasma membranes. I. Chemical composition and enzyme content of plasma membranes isolated from rat liver. *Biocheim. Biophys. Acta.* **90**:126.
24. SNEDECOR, G. W., and W. G. COCHRAN. 1967. Statistical Methods. The Iowa State University Press, Ames, Iowa. 6th edition.
25. WHITTAM, R. 1962. The dependence of respiration of brain cortex on active cation transport. *Biochem. J.* **82**:205.
26. KLEINZELLER, A., and A. KNOTKOVÁ. 1964. The effect of ouabain on the electrolyte and water transport in kidney cortex and liver slices. *J. Physiol. (London).* **175**:172.
27. KAWADA, J., R. E. TAYLOR, JR., and S. B. BARKER. 1969. Measurement of Na-K-ATPase in the separated epidermis of *Rana catesbeiana* frogs and tadpoles. *Comp. Biochem. Physiol.* **30**:965.
28. VALCANA, T., and P. S. TIMIRAS. 1969. Effect of hypothyroidism on ionic metabolism and Na-K activated ATP phosphohydrolase activity in the developing rat brain. *J. Neurochem.* **16**:935.
29. KRISHNA, G., S. HYNIE, and B. B. BRODIE. 1968. Effects of thyroid hormones on adenyl cyclase in adipose tissue and on free fatty acid mobilization. *Proc. Nat. Acad. Sci. U.S.A.* **59**:884.
30. LEVEY, G. S., and S. E. EPSTEIN. 1969. Myocardial adenyl cyclase: Activation by thyroid hormones and evidence for two adenyl cyclase systems. *J. Clin. Invest.* **48**:1663.
31. WISWELL, J. G., K. L. ZIERLER, M. B. FASANO, and S. P. ASPER. 1954. The effects of L-triiodothyronine and L-thyroxine on the metabolism of tissues in vitro. *Bull. Johns Hopkins Hosp.* **94**:94.
32. TOSTESON, D. C., and J. F. HOFFMAN. 1960. Regulation of cell volume by active cation transport in high and low potassium sheep red cells. *J. Gen. Physiol.* **44**:169.