Thyroid Hormone Action: Effect of Triiodothyronine on Mitochondrial Adenine Nucleotide Translocase In Vivo and In Vitro

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Adenine nucleotide translocase (AdNT) levels were measured as the exchange of extramitochondrial against intramitochondrial adenosine diphosphate (ADP) in liver, spleen, and testes mitochondria isolated from normal and hypothyroid rats using the "back-exchange" and atractyloside-stop method of Pfaff and Klingenberg. The results provide confirmation of previous reports that mitochondria from hypothyroid rats show a markedly diminished AdNT activity, which is restored to normal levels within 72 hours by intraperitoneal injection of 10 to 20 μg triiodothyronine (T_3)/100 g body weight. The latter dose was found in dose-response studies to result in maximal stimulation of AdNT in liver mitochondria. Qualitatively similar results on AdNT activity were obtained in liver mitochondria within 30 to 60 minutes following intravenous injection into hypothyroid rats of a more physiological dose of T_3 (40 ng/100 g body weight). AdNT in mitochondria isolated from spleen and testes (organs that do not exhibit a calorigenic response after administration of thyroid hormone to the whole animal) failed to respond to thyroidectomy and to administration of T_3. More recently, we have observed that in vitro replacement of T_3 also stimulates AdNT activity in hypothyroid liver mitochondria. The enzyme adenosine triphosphate (ATP) synthase was examined as another possible candidate for direct hormonal stimulation of mitochondria. Simultaneous determinations on the same rats after intraperitoneal injection of T_3 (20 μg/100 g body weight) showed little or no effect on ATP synthase until after 37 to 85 hours, whereas enhanced activity of the translocator was regularly observed at 17 hours. These findings support the view that AdNT, which is considered to exert major control over the rate of oxidative phosphorylation, may be a direct target of T_3 action on the mitochondria. Increased nuclear transcription may be regarded as a sustained delayed effect of T_3 administration, in contrast to the early effect on mitochondrial AdNT. A bolus intravenous injection of T_3 into the hypothyroid rat increases the activity of the mitochondrial carrier AdNT within a matter of minutes as an early direct effect, as also suggested by studies of addition of T_3 in vitro to isolated rat liver mitochondria. In contrast, nuclear effects require 12 to 24 hours to show increased transcription, evidenced by increased specific mRNA directing the formation of more AdNT (Luciakova and Nelson).

OVER THE LAST 35 years, a number of mechanisms have been advanced to explain the multiple physiological actions of thyroid hormone, ie, stimulation of growth, acceleration of metamorphosis, control of basal metabolic rate, and regulation of protein, lipid, and electrolyte metabolism.[1] In general, the majority of these mechanisms attempt to explain the biological action of thyroid hormone through some form of direct action of the hormone on (1) DNA transcription in the nucleus,[2,3] (2) on hormone receptors of the plasma membrane,[4-6] or (3) the respiratory and phosphorylative machinery in mitochondria.[7-9] A reasonable case has been made for all three sites of action.[10] Several laboratories have reported binding of the active hormone triiodothyronine (T_3)[1] by specific hormone receptors of nuclear chromatin,[11,12] which was recently shown to be the same as the hc-*erb* A protooncogene.[13,14] The occupancy of these nuclear receptors by T_3 is reported to produce increased transcription of genetic messages resulting in more mRNA and increased protein synthesis.

However, increased protein synthesis is unable to account for the rapid increase in oxygen consumption observed with administration of T_3 to hypothyroid animals or to preparations of mitochondria (essentially devoid of nuclear enzymes) in which protein synthesis has been blocked by prior administration of inhibitory drugs such as cycloheximide, puromycin, or actinomycin D.[15] Such findings have raised the question of a cascade of hormonal effects at the cellular level.

Previous research from our laboratory[7] supported specific binding of T_3 to mitochondria of responsive tissues, ie, those that exhibit increased oxygen consumption in response to T_3. The exceptions were the "unresponsive" tissues (adult brain, spleen, and testes) that do not exhibit increased respiratory activity after thyroid hormone administration to the whole animal.[16]

Adenine nucleotide translocase (AdNT) plays a key role in energy metabolism by exchanging adenine nucleotides between the cytosol and the mitochondrial matrix, and in so doing provides the cytosol with adenosine triphosphate (ATP) to drive energy-requiring reactions.[17,18] A series of findings from our laboratory[19] and others[20,21] have led to the suggestion that AdNT, a nuclear-coded protein housed in the inner membrane of mitochondria, is a major target of T_3 action in liver mitochondria and in mitochondria of other T_3-responsive tissues as well. The purpose of this report is to describe in vivo and in vitro experiments that show effects of thyroid hormone on the activity of AdNT in rat liver mitochondria. The findings strengthen the view that AdNT is a key target of T_3 action on the mitochondria.

MATERIALS AND METHODS

Male Spague-Dawley rats (220 to 250 g) of the SD-NIH strain were either purchased postthyroparathyroidectomy or rendered hypothyroid by the addition of 0.05% propylthiouracil to the

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drinking water for 2 to 3 weeks. Thyroidectomized rats were maintained for at least 1 month on a low-iodine diet (Teklad, Madison, WI; with 0.2% $CaCl_2$ added to their drinking water) before killing. No differences were observed between results obtained with the two types of hypothyroid animals. Untreated rats doubled their weight in approximately 8 weeks, whereas treatment with propylthiouracil resulted in virtual cessation of growth. Verification of the thyroid function status of the rats was obtained by T_3 and thyroxine (T_4) radioimmunoassay of the sera collected at the time of death. The values obtained were in line with expectations. Normal rat serum T_4 (n = 18) was 6.53 ± 1.61 μg/dL and serum T_3 was 56.5 ± 24.5 ng/dL (mean ± SD). Serum T_4 and T_3 in the hypothyroid state were below the level of assay sensitivity and therefore could not be measured reliably. Rats killed 30 minutes after injection of 40 ng T_3/100 g body weight exhibited varying T_3 elevations as high as 96 ng/dL and appropriately higher with greater intravenous doses.

Treatment of hypothyroid rats with T_3 was performed by intravenous injection of a single dose into the tail vein or by intraperitoneal injection as described in the legends to tables and figures. Serum levels of thyroid hormones (ie, T_3 and T_4) were monitored by radioimmunoassay. All the data presented were taken from animals whose circulating T_3 and T_4 levels were consistent with their presumed thyroid state. In the in vitro studies where mitochondria from nonresponsive organs were used, it was necessary to pool the organs from two rats to provide sufficient mitochondria for analysis. The organs of two rats in each thyroid state (ie, euthyroid, hypothyroid, hypothyroid + T_3, and the T_3 vehicle [10 mmol/L NaOH]) were also pooled for in vivo experiments entailing the intraperitoneal or intravenous injection of the hormone. Before being killed, the rats were anesthetized by intraperitoneal injection of pentobarbital.

Liver,[22] spleen,[23] and testes[24] mitochondria were prepared from rat organs as previously described. Mitochondrial membranes were prepared by sonication of rat liver mitochondria essentially as described by Catterall and Pedersen.[25] The sonicated membrane fraction was washed once and resuspended in 0.25 mol/L sucrose containing 20 mmol/L Tris-SO_4 to a final concentration of 20 mg/mL. The membrane protein level was measured spectrophotometrically[26] after diluting a sample in concentrated formic acid. The reference cell contained concentrated formic acid.

Translocase Assay

The AdNT level was measured as an exchange of extramitochondrial against intramitochondrial adenosine diphosphate (ADP) using the "back-exchange" and atractyloside-stop method of Pfaff and Klingenberg.[27] In a typical experiment, mitochondria were "loaded" with ^{14}C-ADP by incubating 12.5 mg protein in 0.5 mL loading buffer for 30 to 45 minutes with 44 nmol [^{14}C-ADP] containing 0.5 μCi radioactivity in a final volume of 0.5 mL. After incubation, the mitochondria were washed twice to remove external radioactivity.

Loaded mitochondria (1 mg) were incubated in a 1-mL microfuge tube for 5 minutes at 2°C in a buffer containing T_3 or an equal volume of T_3 vehicle (10 mmol/L NaOH). The buffer contained 100 mmol/L KCl, 50 mmol/L Tris-SO_4, 1 mmol/L EDTA, and 2 mmol/L EGTA. The final pH and volume of the mixture was 7.4 and 625 μL, respectively. Upon addition of unlabeled ADP (25 μL; final concentration, 60 μmol/L), the reaction was vortexed immediately and the incubation continued at 2°C for exactly 1 minute. The reaction was terminated by the addition of 50 μL 1 mmol/L carboxyatractyloside (CAT) (final concentration, 71 μmol/L). The mitochondria were sedimented at 4°C, and the ADP-dependent radioactivity released to the supernatant solution was measured on a 200-μL aliquot. The results were corrected for the efflux of ADP that occurred in the presence of 70 mmol/L CAT. The latter value, which was similar to the efflux that occurred in the absence of added ADP, amounted to 20% to 30% of the ADP-dependent, CAT-sensitive efflux. The back-exchange was calculated from the ADP-dependent release of radioactivity to the extramitochondrial fluid:

$$\text{counts/min}_{ex} - \text{counts/min}_{71\ \mu\text{mol/L CAT}} = \Delta\text{counts/min}_{ex},$$

$$\%\ \text{exchange} = \frac{\Delta\text{counts/min}_{ex}}{\text{total counts/min} - \text{counts/min}_{71\ \mu\text{mol/L CAT}}} \times 100. \quad \text{Eq. 1}$$

Spectrophotometric Determination of ATPase Activity in the Presence of an ATP-Regenerating System

ATPase activity was measured spectrophotometrically at 340 nm by coupling the production of ADP to the oxidation of NADH via the pyruvate kinase and lactic dehydrogenase reactions as described by Pullman.[28] The incubation mixture contained (in a final volume of 1 mL) 50 mmol/L Tris-sulfate, pH 7.4, 1 mmol/L $MgSO_4$, 0.2 mmol/L NADH, 2 mmol/L phosphoenol pyruvate, 2 mmol/L ATP, pH 7.4, 5 mmol/L KCN, and 1 U each of lactic dehydrogenase and pyruvate kinase. A mixture of all reagents excluding ATP and KCN was prepared and added as a single addition to a 1.3-mL quartz cell. After addition of KCN and the mitochondria, ATP was added and the oxidation of NADH was determined for 3 minutes in a 552A Perkin-Elmer (Norwalk, CT) recording spectrophotometer. Except for the first 30 seconds, the reaction followed zero-order kinetics.

Determination of ATPase Activity by Phosphate Released in the Absence of an ATP-Regenerating System

Intact mitochondria. In this procedure, the release of P_i from ATP was measured. The incubation mixture contained (in a final volume of 1 mL) 50 mmol/L Tris-sulfate, pH 7.4, and 5 mmol/L ATP, pH 7.4. After a 5-minute equilibration at 30°C, 0.5 to 1.5 mg mitochondrial protein was added and the incubation continued for an additional 10 minutes. The reaction was stopped with 0.5 mL 50% trichloroacetic acid. After centrifugation of the precipitated protein, 0.5 mL of the supernatant was analyzed for P_i.[29]

Mitochondrial vesicles. The procedure was essentially the same as for intact mitochondria except that 3 mmol/L $MgSO_4$ was included in the incubation mixture.

Other Methods

Oxygen uptake (state 4)[30] was measured polarographically with a Clark-type platinum electrode.[31] The incubation mixture contained (at pH 7.4) 210 mm D-mannitol, 70 mmol/L sucrose, 1 mmol/L HEPES, 1 mmol/L EDTA, 10 mmol/L P_i, 2.5 mmol/L $MgSO_4$, 6 μg rotenone, 10 mmol/L succinate, 5 U hexokinase, 0.3 mmol/L glucose, and 2.5 mg mitochondria. State 3 respiration was initiated by addition of ADP (final concentration, 1.7 mmol/L).

Definition of Unit and Specific Activity

A unit of activity is defined as the amount of enzyme required to turn over 1 μmol substrate per minute under the specified assay conditions. Specific activity is expressed as units per milligram of protein.

Treatment of Data

Statistical significance was determined by Student's *t* test. Significance is expressed compared with the hypothyroid control. Unless otherwise stated, each entry is the mean ± 1 SEM.

RESULTS

The correlation between adenine nucleotide translocation and oxygen consumption is illustrated graphically in Fig 1, which shows no overlap between the two thyroid states. The excellent correlation is quite compatible with AdNT as a determinant of the rate of oxidative phosphorylation, which is considered a necessary but insufficient line of evidence.

The demonstration by Babior et al[32] and Portnay et al[33] of diminished AdNT activity in liver mitochondria isolated from hypothyroid rats and the restoration of the activity to euthyroid levels by administration of T_4 raised the possibility that the calorigenic effect of thyroid hormone might be exerted through this carrier. Since hormone treatment consisted of daily intraperitoneal injections administered over 6 days, it was not clear how rapidly the changes in AdNT activity occurred. The initial calorigenic effect of intravenous T_3 is known to occur quite rapidly, ie, within an hour or 2 after T_3 administration.[34] Accordingly, we decided to examine this rather important point.

In an attempt to determine the approximate sequence of events in terms of translocase activity, we used the same large T_3 dose (20 μg/100 g body weight) used by Babior et al.[32] Their data had shown a statistically significant increase in AdNT activity only after 72 hours following T_3 administration. Accordingly, we injected three pairs of rats intraperitoneally with T_3 (20 μg/100 g body weight). At 20, 44, and 120 hours after injection, a pair of rats were killed and the livers pooled before homogenization and isolation of the mitochondria. The results were compared with those for hypothyroid "vehicle"-injected controls and are summarized in Fig 2. It is evident on inspection that AdNT activity was increasing up to 120 hours after a single intraperitoneal injection of a very large dose of T_3. Moreover, the earliest time point (20 hours) showed an appreciable increment above the hypothyroid vehicle controls.

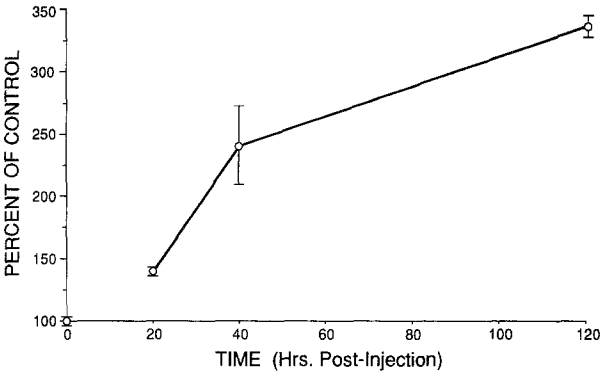

Fig 2. Time course of intraperitoneal T_3 effect on AdNT activity. Pairs of rats were injected intraperitoneally with either 20 μg T_3/100 g body weight or vehicle and killed at the times indicated. AdNT activity was measured. The ordinate values represent the percentage of the control (ie, vehicle-injected hypothyroid rats) AdNT activity. Error bars represent ±1 SEM. The first two points have too small SEM values to be depicted by the figure. The values were 100% ± 1.9% for the control, increasing to 140% ± 1.9% 20 hours after the intraperitoneal T_3, an increase that is highly statistically significant ($P < .001$) by Student's t test.

Before starting intravenous studies to elucidate the onset of this effect, it seemed reasonable to obtain a dose-response relation. We used an intraperitoneal injection of 20 μg T_3/100 g body weight followed by killing at 72 hours.

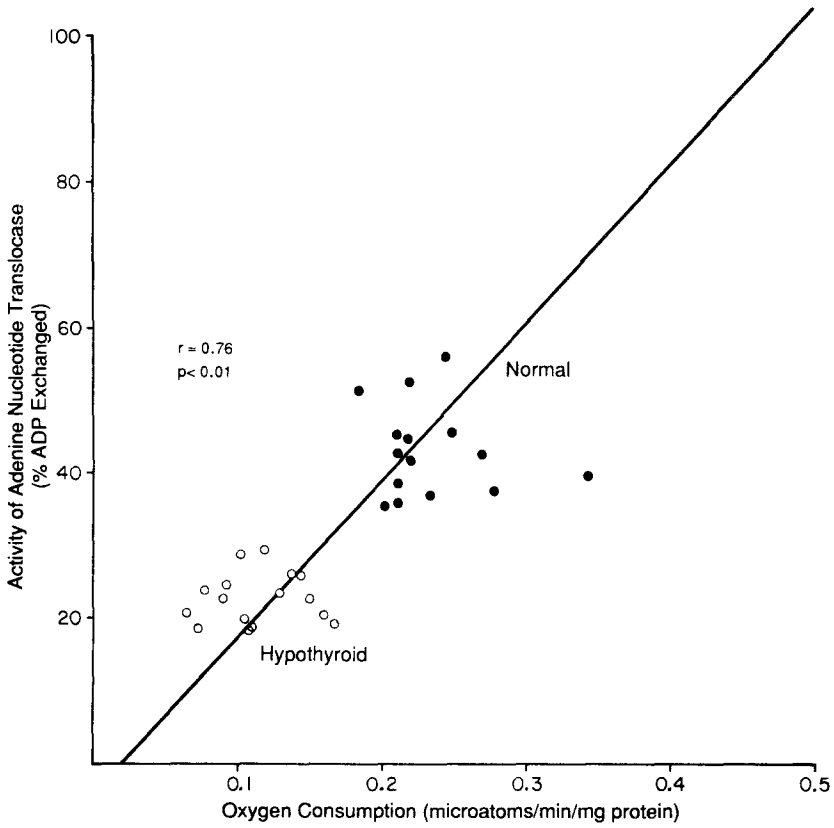

Fig 1. Relation between AdNT activity and oxygen consumption in mitochondria from normal and hypothyroid rats. With both groups correlation is significant, but not within either alone.

Owing presumably to inherent variability, it required 42 rat studies to obtain the approximate relation depicted in Fig 3. As discussed below, the 72-hour response probably reflects more than a single factor.

To seek an early response, we used the intravenous route with the "near-physiological" dose of 40 ng/100 g body weight. A small but consistent stimulation was observed (Fig 4).

Other studies were designed to demonstrate hormonal stimulation of mitochondria in vitro. After preliminary inconclusive efforts (not shown), we kept the mitochondria at 2°C as had been done for all AdNT assays. A 5-minute preincubation of the mitochondria with 30 nmol/L T_3 at 2°C immediately before the addition of unlabeled ADP to initiate the exchange resulted in a partial restoration of AdNT activity (Fig 5). The concentration of T_3 required to show an in vitro stimulation was appreciably higher than the mean concentration in normal rat serum.[35,36] The stimulation at such a short interval suggested to us some physical chemical effect of the hydrophobic T_3 on the protein carrier known to form an association with lipid of the inner membrane.[18] The rather consistent but modest increment in carrier activity was viewed as compatible with early onset of hormonal activation.

Of special interest was the question of the nonresponsive organs (spleen, testes, and adult brain) that had been found to have no calorigenic effect from injected thyroid hormone in the classic studies of Barker and Klitgaard.[16] Pairs of hypothyroid rats were administered intraperitoneal injections of 20 μg T_3/100 g body weight and killed after 72 hours. The organs (liver, spleen, and testes) from each pair of rats were pooled before homogenization and isolation of mitochondria. Pronounced reduction in liver AdNT was evident in the hypothyroid rat liver as compared with the normal, as well as restoration of AdNT to above-normal levels in hypothyroid rats that received the large T_3 dose (Table 1). In contrast, no clear alteration was evident in spleen or testes mitochondria from the same animals—that is, no effect of thyroid function status or T_3 injection.

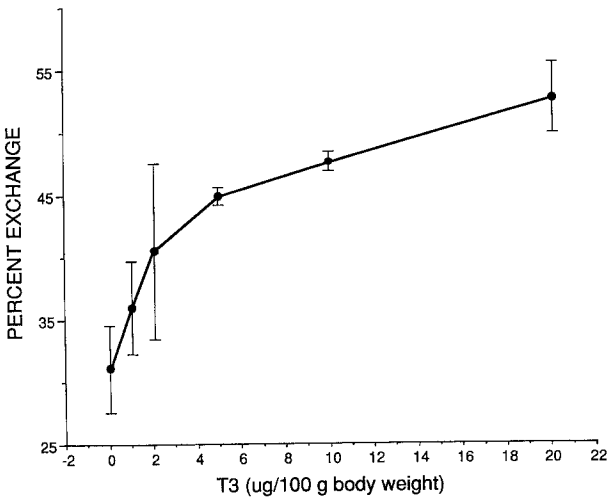

Fig 3. Response of AdNT activity to dose of T_3. The experiments were performed as described in Fig 1, except that the rats were killed 72 hours after the indicated dose of T_3 was administered.

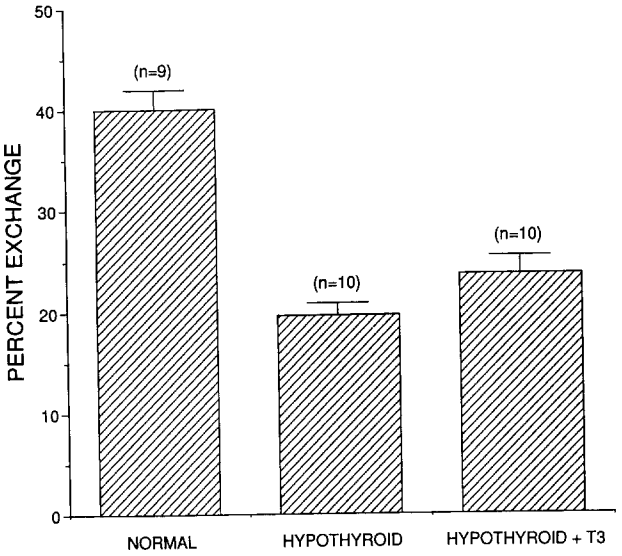

Fig 4. Rapid intravenous effect of T_3 on AdNT activity. Rats were rendered hypothyroid. Pairs of hypothyroid rats were injected in the tail vein either with vehicle (10 mmol/L NaOH) or with vehicle containing 40 ng T_3/100 g body weight. The thyroid status of each animal was verified by radioimmunoassay of serum T_3 and T_4. Thirty minutes after injection of T_3, the rats were killed and the mitochondria isolated. AdNT activity was measured. Numbers in parentheses refer to the number of experiments. Each entry is the mean ±1 SEM. Differences between normal and hypothyroid and between hypothyroid and hypothyroid + T_3 are significant by Student's *t* test at $P < .001$ and $P < .025$, respectively.

It is widely believed that AdNT is a major regulator of ATP production in mitochondria. This effect is mediated by its ability to supply ADP to the ATP synthase enzyme, considered unsaturated with respect to its substrate ADP. According to this model, stimulation of respiration by

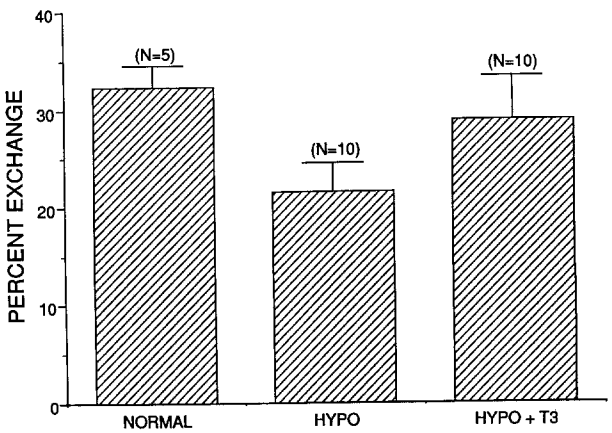

Fig 5. Effect of T_3 in vitro on AdNT activity. Isolated mitochondria were loaded with [^{14}C]-ADP. Before assay for AdNT activity, the loaded mitochondria were incubated for 5 minutes at 2°C with 30 nmol/L T_3 or an equal volume of vehicle. Each entry is the mean ± 1 SEM. Differences between normal and hypothyroid and between hypothyroid and hypothyroid + T_3 are significant by Student's *t* test at $P < .001$ and $P < .01$, respectively.

Table 1. Effect of T_3 on AdNT Activity in Rat Liver, Spleen, and Testes Mitochondria

	Liver	Spleen	Testes
Normal	49.9 ± 2.4(16)	38.6 ± 0.75 (5)	51.6 ± 5.50(3)
Hypothyroid	31.1 ± 3.2(12)	44.0 ± 6.42 (4)	43.3 ± 3.42(3)
Hypothyroid + T_3	52.6 ± 2.8(11)	47.5 ± 10.51(3)	38.3 ± 3.39(3)

NOTE. Measurements of AdNT activity were made on mitochondria isolated from pairs of normal, hypothyroid, and hypothyroid rats that received an intraperitoncal injection of 20 μg T_3/100 g body weight 72 hours before killing. In each experiment, the mitochondria were prepared from two rat organs that were pooled before homogenization. Numbers in parentheses refer to the number of experiments. Each entry is the mean ±1 SEM. Differences among groups of the liver mitochondria (normal *v* hypothyroid and hypothyroid *v* hypothyroid + T_3) were highly statistically significant ($P < .001$). In contrast, differences between groups from the unresponsive organs were not significant.

thyroid hormone would arise indirectly as a result of increased activity of both AdNT and ATP synthase. Accordingly, we initiated studies to determine whether ATP synthase responds to the hormonal state of the animal as does AdNT. Since ATP synthase works in both hydrolytic and synthetic directions, measurement of the hydrolytic activity was taken as a measure of the ATP synthase activity of the mitochondrial preparations. ATPase activity was measured in the presence and absence of CAT and in the presence and absence of oligomycin. Over 95% of the activity was oligomycin-sensitive, and thus mitochondrial in origin. Approximately 60% of the activity was CAT-sensitive and thus dependent on AdNT activity. Hypothyroid mitochondria invariably showed a significant decrease in total, as well as CAT-sensitive, ATPase activity (Table 2). In general, mitochondria from hypothyroid rats injected with T_3 showed increases in ATPase activity that correlated with increases in AdNT activity. However, the correlation was only noted after 37 to 85 hours, whereas increases in AdNT were regularly observed after 17 hours.

DISCUSSION

After we had demonstrated the more than threefold increase in AdNT activity (from hypothyroid to hyperthyroid level) 5 days after a large intraperitoneal T_3 injection (Fig 2), we then approached the question of an effect not likely attributable to stimulation of transcription and protein synthesis. The small but significant increments 30 minutes after intravenous T_3 in a physiological dose (Fig 4) and 5 minutes after incubation in vitro (Fig 5) afford support for our inference of an incipient direct hormonal action on the translocator.

In a recent report, Rasmussen et al[36] concluded from affinity-labeling experiments using the bromoacetyl analog of T_3 that AdNT is not the high-affinity binding component in the inner mitochondrial membrane. However, photoaffinity experiments reported from our laboratory,[37] in which underivatized [^{125}I]-T_3 was used, identified AdNT as the covalently labeled protein on two-dimensional electrophoresis. Indeed, the alkylating bromoacetyl side chain rather than T_3 would appear to determine the labeling, in this case the enzyme creatine kinase, according to the evidence and discussion presented by Wyss et al.[38] The more recent data would appear to refute completely the conclusions of Rasmussen et al.[36]

The concept of a physical chemical mode of direct activation of mitochondria by T_3 must be regarded as speculative in the absence of precise mechanistic information as to how this might occur. However, the present findings are not the first to suggest rapid hormone action on mitochondria by a pathway not involving the cell nucleus or transcription. The report of Mowbray and Corrigall[39] showed effects on the AdNT K_m 15 minutes after intravenous T_3 injections into hypothyroid rats. In studies in vivo and others using the isolated perfused rat liver, Seitz et al[40] showed changes in ADP to ATP ratios within 2 hours, which they attributed to increased mitochondrial adenine nucleotide transport with associated increased mitochondrial respiration. In a more recent study from the same laboratory by Höppner et al,[41] evidence was presented suggesting that enhanced ADP/ATP transport resulting in increased mitochondrial metabolic activity was not associated with increased gene expression. This inference was based on a study of Northern blots of heart, liver, and kidney mRNA of translocator AdNT, as well as sodium dodecyl sulfate–polyacrylamide gel electrophoresis study of the mitochondrial proteins, which showed no clear differences between normal, hypothyroid, and hyperthyroid rats. This significant finding awaits independent confirmation.

In a recent study from Cambridge, UK, Hafner et al[42] have used a kinetic method in examining T_3 effects on mitochondria to determine whether the respiratory chain or the phosphorylation system is the site of hormone stimulation. These investigators view oxidative phosphoryla-

Table 2. Effect of Hormonal State on ATPase and AdNT Activity

	ATPase (corrected) Activity as % of Hypothyroid Vehicle Control				AdNT Activity as % of Hypothyroid Vehicle Control				
Experiment No.	Normal	17 h	37 h	41 h	Normal	17 h	37 h	41 h	85 h
54	141	105			150				
55	164	98	149		153	179	216		
56	279	100			192	141			200
57		100		189			167		
58		105		197					

NOTE. Hypothyroid rats were killed at various intervals after intraperitoneal injection of either vehicle or T_3 (20 μg/100 g body weight). The ATPase level was measured spectrophotometrically in the presence and absence of CAT. Results are expressed as the percentage of activity found in the vehicle-injected hypothyroid rat. AdNT activity was measured as described and is also expressed as the percentage of activity found in the vehicle-injected hypothyroid rat.

tion as divisible into two groups of reactions: (1) those that generate proton motive force (Δp), which include the respiratory chain, and (2) those that consume Δp, which include AdNT and ATP synthase. The data of these researchers showed that at any given mitochondrial membrane potential, the respiration rate was markedly greater in euthyroid than in hypothyroid mitochondria. This finding, together with theoretical considerations, suggested thyroid hormone action on ATP synthase and/or AdNT. Of these two candidates, AdNT was regarded as primarily affected by thyroid hormone stimulation. Our findings showed that ATP synthase was enhanced only after 37 to 85 hours, whereas the translocator activity was regularly increased 17 hours after T_3 injection, in simultaneous studies on the same rats.

The most significant finding of the present report is the observation of AdNT stimulation by T_3 30 minutes after intravenous injection, as well as the hormonal stimulation of the mitochondria in vitro. These phenomena support the concept of a direct pathway for early AdNT activation, which is believed to precede the cascade of major biochemical events, including new protein formation, not evident until many hours after a bolus intravenous T_3 injection.

In the absence of clear and incontrovertible evidence regarding the precise role of the putative mitochondrial pathway in the cascade of thyroid hormone action in relation to nuclear and extranuclear events, it is appropriate to summarize briefly several lines of evidence that suggest direct T_3 stimulation of mitochondrial oxidative phosphorylation:

1. Accumulation of labeled T_3 by mitochondria of dispersed rat hepatocytes, more than any other organelle.[43]
2. Increased oxygen consumption in hepatocytes incubated with T_3 as compared with controls, a stimulation that persisted despite cycloheximide blockade of protein synthesis.[44]
3. Specific (ie, displaceable) binding of T_3 with high affinity ($K_a > 10^{11}$ mol/L^{-1}) by a component of the mitochondrial inner membrane[7] from rat liver mitochondria, but not from the mitochondria of spleen or testes (recognized as organs that lack a calorigenic response to thyroid hormone[16]). Of special interest were the findings in neonatal rats that exhibited specific brain mitochondrial binding in the first 2 weeks of life (when the brain does indeed show calorigenic effects of hormone). However, both the calorigenic effect of T_3 and its specific binding are lost after the first 2 weeks of life. Moreover, studies with hormone analogs have shown biological activity proportional to affinity of binding of the analogs to the inner membrane protein.[7]
4. Rapid (30-minute) stimulation of depressed mitochondrial oxygen consumption and ATP formation in hypothyroid rats treated with intravenous bolus T_3 injection in the nanogram range.[15]
5. Identification of the inner mitochondrial membrane T_3 binding protein as AdNT.[37]
6. AdNT activity found to be markedly diminished in the hypothyroid rat, but restored by hormone replacement, with early effects apparent within 30 minutes of intravenous T_3 injection (current report).
7. Rapid stimulation of hypothyroid AdNT activity by 5-minute incubation of mitochondria with T_3 in vitro (current report).

Our present speculation is that the phenomena studied seem to be initiated by a rapid direct physical-chemical interaction between hormone and receptor, resulting in prompt increased oxidative phosphorylation. This direct mitochondrial activation provides excess energy well beyond that required for enhanced nucleic acid and protein synthesis.

The recent report[45] by Luciakova and Nelson from Stockholm provides an intriguing picture of the successive effects of thyroid hormone stimulation on mammalian mitochondria. These investigators studied the mRNA levels before and after injections of 20 μg T_3/100 g body weight, as followed by quantitation of the mRNAs directing formation of some nuclear encoded mitochondrial proteins. Incipient increases were seen in 12 hours and maximal effects in 72 hours after T_3 administration. Cytochrome c_1 mRNA and AdNT mRNA were, respectively, the most marked increases observed among Poly(A)-rich mRNAs examined. The investigators recognize enhanced mitochondrial respiration after T_3 injection occurs "long before mitochondrial protein synthesis" is increased, in accordance with our views.

Future investigation will hopefully clarify the mechanism of nuclear and extranuclear hormone action, including the possibility of "cross-talk" among cellular organelles.

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