Intracellular Conversion of Thyroxine to Triiodothyronine Is Required for the Optimal Thermogenic Function of Brown Adipose Tissue

Antonio C. Bianco and J. Enrique Silva
Howard Hughes Medical Institute Laboratory, Brigham and Women's Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115

Abstract

The effect of thyroxine (T_4) and triiodothyronine (T_3) on the expression of uncoupling protein (UCP) in rat brown adipose tissue (BAT) has been examined. Thyroidectomized rats have a threefold reduction in basal UCP levels. When exposed to cold, they become hypothermic and show a fivefold lower response of UCP than euthyroid controls. T_3 augments the basal levels and the response of UCP and its mRNA to cold in a dose-dependent manner. However, to normalize the response of UCP, T_3 has to be given in a dosage that produces systemic hyperthyroidism. Mere T_3 replacement corrects the systemic hypothyroidism but not the hypothermia or the low levels of UCP. In contrast, replacement doses of T_4 prevent the hypothermia and correct the UCP level. Both effects of T_4 are blocked by preventing T_4 to T_3 conversion in BAT. Thus, the optimal UCP response to cold and protection against hypothermia require a high BAT T_3 concentration, which is attained from euthyroid levels of T_4 via the activation of the BAT T_4 5'deiodinase during cold exposure, but not from euthyroid plasma T_3 levels.

Introduction

Brown adipose tissue (BAT)[1] is a site of facultative nonshivering thermogenesis where heat production increases markedly in response to low ambient temperature and overfeeding (diet-induced thermogenesis) by mechanisms triggered by the sympathetic nervous system (1–3). The cornerstone of these responses is the capacity of BAT to increase the oxidation of fatty acids and, at the same time, to uncouple oxidative phosphorylation. This latter is accomplished by a 32-kD protein located in the inner membrane of mitochondria called thermogenin or uncoupling protein (UCP). This protein is unique to BAT and considered to be a rate-limiting factor for the thermogenic function of this tissue (3). Thyroid hormone has also a well recognized thermogenic effect, but this is believed to result largely from the widespread metabolic stimulation in other tissues rather than in BAT, where it would just play a permissive role (4, 5). However, BAT contains a thyroxine (T_4) 5'deiodinase (5'D), which catalyzes the conversion of T_4 to triiodothyronine (T_3), 10 times more potent than T_4 (6). This enzyme is markedly activated by sympathetic stimulation (7, 8), which results in a several-fold increment in the concentration of T_3 in BAT with no major change in plasma levels of T_4 or T_3 (8). Moreover, we have recently found that BAT has the potential to respond to T_3 in that it contains nuclear T_3 receptors in number comparable with the pituitary and liver, both highly sensitive to thyroid hormone. Over 50% of the nuclear-bound T_3 is generated via BAT 5'D, resulting in an overall receptor saturation of ~70% (9). These findings suggest a critical role for thyroid hormone and hence for BAT 5'D in the function of the brown adipocyte. Accordingly, we have examined in rats the effect of thyroid hormone, specifically the intracellularly generated T_3, on the response of UCP to cold stress. We have found that T_3 is critical for the full response of UCP to cold and that BAT 5'D is essential in providing the appropriate BAT T_3 content at euthyroid plasma levels of T_4 and T_3.

Methods

Animals. The experiments were performed in Sprague-Dawley rats (Zivic-Miller, Allison Park, PA). Thyro-parathyroidectomy with parathyroid reimplant was performed by the supplier. Experiments were performed 3–4 wk after surgery.

Analytical procedures. Mitochondria were isolated from BAT by standard techniques of subcellular fractionation. UCP was purified from cold-acclimated rats following methods previously published (10, 11). The identity of purified UCP was confirmed by its ability to bind guanosine diphosphate. Proteins were analyzed slab polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS PAGE) (12). Rabbits were immunized with purified UCP. Antisera were analyzed by immunoblotting after electrotransfer onto nitrocellulose filter in 200 mM glycine, 20% methanol, and 25 mM Tris buffer (pH 8.3). Parallel gels were stained with Coomassie Blue. Immunoblotting was performed with 1/100 UCP antisera overnight at 4°C followed by a 2-h incubation with goat anti-rabbit gamma globulin conjugated to horseradish peroxidase. Immunocomplexes were visualized with diaminobenzidine and H_2O_2. A solid-phase immunoassay was set up in nitrocellulose wells (Millititer^R plates, Millipore Corp., Bedford, MA). The antigen, either purified UCP or crude mitochondrial protein, was diluted with 150 mM NaCl, 10 mM EDTA, and 50 mM Tris buffer (pH 8.6) and immobilized overnight at 4°C in multiple nitrocellulose well plates (Millititer^R, Millipore Corp.) in a volume of 20 μl/well. The wells were washed and the nonspecific binding blocked with 2% gelatin/0.1% Tween 20 for 2 h. After rinsing, the wells were incubated overnight with a 1/200 dilution

Address correspondence to Dr. Silva, Brigham and Women's Hospital, Department of Medicine, Howard Hughes Medical Institute Laboratory, George W. Thorn Building, 20 Shattuck Street, Room 1009, Boston, MA 02115.

Received for publication 20 August 1986.

1. *Abbreviations used in this paper:* 5'D, 5'deiodinase; αGPD, α glycero phosphate dehydrogenase; BAT, brown adipose tissue; BW, body weight; IOP, iopanoic acid; PAGE, polyacrylamide gel electrophoresis; T_3, triiodothyronine; T_4, thyroxine; Tx, thyroidectomized; UCP, uncoupling protein.

J. Clin. Invest.

0021-9738/87/01/0295/06 $1.00
Volume 79, January 1987, 295–300

of UCP antiserum at 4°C. The amount of antibody bound to the wells was quantitated with ^{125}I-labeled Staphylococcus protein A in a 2-h incubation (13). The results are expressed as micrograms of UCP per milligram of mitochondrial protein. Immunoprecipitation of ^{35}S-labeled UCP labeled from in vivo injections of [^{35}S]methionine or in vitro translated mRNA was carried as described (14) with minor modifications. Equal aliquots of mitochondria (after in vivo labeling) or of TCA-insoluble ^{35}S counts (in vitro translation) were incubated overnight with a 1/100 dilution of UCP antiserum in 10 mM phosphate buffer (pH 7.5) containing 150 mM NaCl, 1% deoxycholate, and 1% Triton X-100 (14). The immunocomplexes were precipitated with goat anti-rabbit IgG for 2 h at 4°C and recovered by centrifugation for 20 min at 2,000 *g* through a cushion of 1 M sucrose. The pellets were washed, counted or analyzed by SDS PAGE, and autoradiographed. Polysomal RNA was prepared as described previously (15) in the presence of 10 mM vanadyl ribonucleosides complexes. Polysomes were resuspended in 4 M guanidinium isothiocyanate and RNA was recovered by centrifugation through 5.7 M CsCl (15). Poly A RNA was isolated with a commercial Poly-U-coated paper, Hybond^{R}-mAP (Amersham Corp., Arlington Heights, IL) as described elsewhere (16). The yield was ~10 μg/g tissue. 1 μg of polysomal mRNA was translated in a rabbit reticulocyte lysate (Bethesda Research Laboratories, Gaithersburg, MD) in the presence of carrier-free [^{35}S]methionine, as described by Pelham and Jackson (17) and with the modifications introduced by the manufacturer of the kit. BAT 5'D and α-GDP activities were measured as described elsewhere (6, 18, 19).

Experiments. Details pertaining to specific protocols are given with the results. Animals were placed at 4°C for 19 or 48 h with water and food ad libitum, with the same light/dark cycle as the controls at 25°C. T_3 or T_4 were injected subcutaneously in 10% rat serum in sterile saline. Iopanoic acid (IOP), 5 mg/100 g body weight (BW) per d, was injected intraperitoneally dissolved in alkalinized saline (pH 10–11) (20); the first injection preceeded the T_4 treatment by 12 h. Controls received the appropriate vehicles. Core body temperature was measured with a thermistor introduced ~5 cm in the rectum. In one experiment ^{125}I-T_4 was injected to assess the effectiveness of IOP to inhibit T_4 to T_3 conversion in vivo; the injection, isolation of BAT cell nuclei, and extraction and analysis of the ^{125}I-T_3 were performed as described previously (9). Statistical analysis of the results included one-way analysis of variance and multiple comparison using the RS/1 programs of the BBN Instruments Corp. (Cambridge, MA).

Results

Purification of UCP, antisera, and immunoassay. The yield of UCP was ~70 μg/g of BAT. One out of four rabbits immunized with two injections of 50 μg of UCP gave a highly specific antibody that has been used in the present experiments. Fig. 1 *A* illustrates the degree of purification of UCP and the presence of a prominent 32-kD band in mitochondria (lanes *4* and *5*), but not in cytosol. The immunoblotting of these proteins, after transfer to nitrocellulose paper (Fig. 1 *B*), identifies the 32-kD band as UCP and illustrates the specificity of the antibody. The assay was highly specific and sensitive; it could detect 50 ng of UCP, which allowed us to assay as little as 1–2 μg of mitochondrial protein (see Fig. 2).

Effect of thyroid hormones on body temperature and UCP levels in rats maintained in the cold for 48 h. Intact rats maintained their core temperature after 48 h at 4°C, whereas the hypothyroid animals had severe hypothermia (Table I). At 25°C, the UCP level in hypothyroid animals was about one-third of that of intact rats (10 vs. 32 μg/mg) and, although in both groups increased significantly with the cold exposure, the level was four to five times higher in the euthyroid than in the hypothyroid rats (76 vs. 17 μg/mg; Table I). Groups of thyroidectomized (Tx)

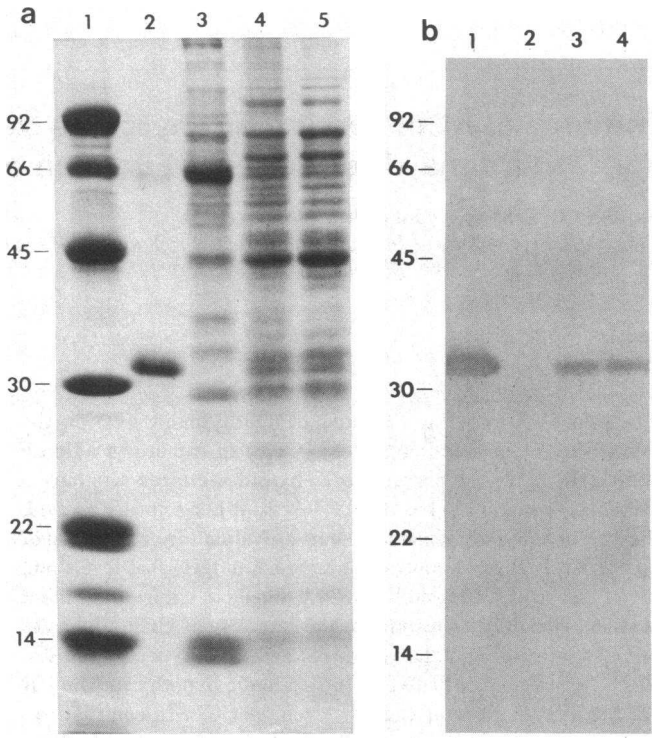

Figure 1. (*a*) SDS PAGE of purified UCP (lane *2*), BAT cytosol (lane *3*), and BAT mitochondria fraction either treated (lane *4*) or not (lane *5*) with 3.2% lubrol. (*b*) Immunoblotting of the gels shown in *A* with our rabbit anti-rat UCP antiserum after electrotransfer onto nitrocellulose paper. Lane *1*, purified UCP; lane *2*, cytosol; lane *3*, lubrol-treated mitochondria; and lane *4*, crude mitochondria.

rats were treated with T_3, 150 ng/100 g of BW subcutaneously twice daily for 5 d. This dosage replaces the daily production rate of T_3, normalizes serum T_3 concentration, and restores growth rate and liver enzymes in Tx rats (21). This regimen of T_3 did not prevent the cold-induced hypothermia, and did not correct the UCP concentration either at room temperature (increased only to 17 μg/mg) or in the cold (increased only to 27 μg/mg, one-third of the response of intact animals). Note that T_3 normalized the mitochondrial liver enzyme αGPD (Table I), a sensitive marker of thyroid hormone action in this organ (18),

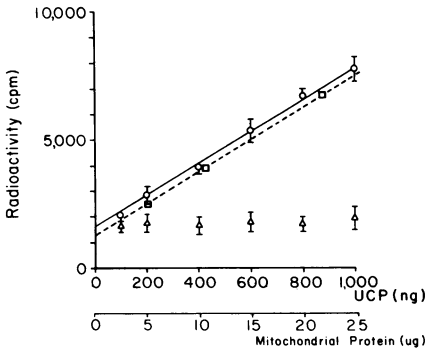

Figure 2. Solid-phase immunoassay of UCP. The ordinate represents the amount of ^{125}I-labeled protein A bound to the wells. Purified UCP (*open circles*) and crude mitochondrial protein (*squares*) generated parallel lines; preadsorption of the antiserum with 100 μg of UCP (*triangles*) prevented the binding.

Table I. Effect of 48 h of Exposure to 4°C on Mitochondrial Levels of UCP in Intact and Tx Rats Treated with T_3 or T_4, with or without IOP

Treatment groups	Temperature	UCP	Body temperature	Liver αGPD
	°C	μg/mg protein	°C	ΔOD/min × 10^2
Intact	25	31.8±2.1	37.9±0.1	8.3±0.7
Tx	25	10.0±0.5	37.7±0.2	3.1±0.4
Tx + T_3	25	17.0±0.9	—	9.3±0.4
Intact	4	75.6±8.7	37.6±0.1	8.7±0.5
Tx	4	17.2±0.8	29.0±3.5‖	2.3±0.3
Tx + T_3	4	27.4±1.3*	35.6±0.5‖	8.9±0.8
Tx + T_4	4	63.4±6.9	37.0±0.3	5.2±0.6
Tx + T_4 + IOP	4	26.0±2.3‡	34.9±0.3‖	3.0±0.5
Tx + T_4 + T_3	4	58.4±4.3	—	9.2±0.5
Tx + T_4 + T_3 + IOP	4	32.7±2.5§	—	8.6±0.7
F		379.4	106.0	215.4

Values are the means±SD of four to five animals. Tx rats were treated with T_3 as described in Fig. 2. T_4, 400 ng/100 g BW, was given every 12 h while the rats were in the cold. IOP, 5 mg/100 g BW per d, was injected intraperitoneally starting 12 h before the first injection of T_4. Cold exposure lasted 48 h. Body temperature was measured with an electronic probe inserted ~5 cm in the rectum. Liver αGPD was measured as described previously and expressed as OD units/min per mg of protein. Intact, Tx + T_4, and Tx + T_4 + T_3 at 4°C were not different. UCP and αGPD in Tx at 25°C was significantly lower than in the intact controls ($P < 0.001$); core temperature was not different. One-way analysis of variance: * $P < 0.01$ vs. Tx at 4°C; ‡$P < 0.01$ vs. Tx + T_4 at 4°C; §$P < 0.01$ vs. Tx + T_4 + T_3 at 4 C; ‖$P < 0.01$ vs. intact rats.

Table II. Effects of Various Doses of T_3 on Mitochondrial Levels of UCP in Thyroidectomized (Tx) Rats After 48 h of Cold Exposure

Dose T_3	UCP	Serum T_3	Liver αGPD
μg/100 g BW per d	μg/mg protein	ng/ml	ΔOD/min × 10^2
0.0	8.0±0.4	0.08±0.02	3.3±0.5
0.3	10.6±0.5	0.59±0.04	8.9±0.4
0.5	11.7±0.9	1.13±0.08	10.7±1.3
0.8	12.4±1.0	1.74±0.10	12.4±0.9
1.3	15.3±1.1	2.77±0.40	14.1±0.3
2.1	23.4±1.5	4.66±0.66	16.4±1.0
4.7	41.6±3.7	10.72±2.10	19.6±2.3
Intact	75.6±8.7	0.67±0.08	8.7±0.5
F	202.9	256.6	63.9

Values are the means±SD of four animals. Tx rats were injected twice daily with either 150 μg T_3/100 g BW or vehicle (top group) for 5 d; they were then segregated in seven groups and given the daily doses of T_3 indicated in the table divided in two subcutaneous injections. This treatment lasted two consecutive days while the rats were maintained at 4°C. Serum T_3 was measured in unextracted serum by radioimmunoassay (14). For all three variables the response to the lowest dose of T_3 was significant ($P < 0.01$).

as it normalized serum T_3 (not shown but see Table III for a similar experiment). In contrast, an amount of T_4 equal to the production rate (7), 400 ng/100 g BW subcutaneously twice daily, just during the 48 h of the cold stress, resulted in a UCP level of 63 μg/mg, statistically not different from the response in the euthyroid animals, and prevented the hypothermia. This regimen of T_4 did not correct the systemic hypothyroidism because it did not normalize either serum T_4, T_3, or liver αGPD. IOP, a potent competitive inhibitor of 5'D (20), abolished the correction by T_4 of both, the UCP levels and hypothermia. The effectiveness of IOP to block T_4 to T_3 conversion was confirmed with ^{125}I-T_4 injected to a parallel group of animals. IOP decreased nuclear ^{125}I-T_3 by >90% without affecting plasma or BAT ^{125}I-T_4 concentrations, and abolished the activity of the BAT 5'D.

Response of UCP to various doses of T_3. To understand better the quantitative relationships between the UCP responses and BAT T_3, Tx rats were treated for 5 d with 0.15 μg T_3/100 g BW subcutaneously twice a day and then placed them in the cold for 48 h. At this time, they were segregated in groups of four rats which were injected subcutaneously with various doses of T_3 at ~12-h intervals. The results are shown in Table II. The highest dose, 4.7 μg/100 g BW per d, elevated the post-cold level of UCP from ~8, in the untreated rats, to 41 μg/mg of mitochondrial protein, a response still lower than in euthyroid animals or in Tx rats treated with T_4 for 48 h where UCP level reached ~70 μg/mg (Table I). In contrast, the lowest dose of T_3 normalized the liver αGPD and serum T_3 concentration, whereas higher doses resulted in hyperthyroid serum levels of T_3 and αGPD activity. Actually, the highest dose of T_3 provided an average nuclear receptor saturation of >90%, as can be calculated from the half-saturating plasma T_3 concentration (9) and the integrated plasma T_3 concentration obtained from the serum T_3 level in Table II and the half-time of disappearance of T_3 from plasma in cold-exposed rats (8).

Effects of acute cold exposure and thyroid hormones on the endogenous labeling of UCP with [^{35}S]methionine in hypothyroid rats. To determine whether the effect of thyroid hormone was due to increased synthesis of UCP, Tx rats were treated with T_3 as above and placed in the cold. Other animals were injected with a single intraperitoneal dose of 800 ng T_4/100 g BW, either alone or in addition to the T_3 regimen, at the time of starting the cold stress. The total duration of the cold stress was 19 h. After 16 h in the cold, the rats were injected intravenously with ~300 μCi of [^{35}S]methionine and killed 3 h later. The immunoprecipitates of mitochondrial proteins were analyzed by SDS PAGE and autoradiography. The autoradiograms showed only one 32-kD band of radioactivity in all the experimental conditions (Fig. 3 *A*). The intensity of this band, which represents the fraction of ^{35}S counts incorporated into protein as UCP, was enhanced by cold exposure. This response was only slightly improved by T_3 but markedly enhanced after one dose of T_4. Table III presents a more detailed and quantitative account of this experiment. Results are expressed as the mean±SD percent of the protein-bound ^{35}S recovered in the immunoprecipitate in the individual rats. While T_3 augmented the response to cold by ~90% over the untreated animals ([4.73–2.45]/[3.62–2.45]), a single injection of T_4 amplified fivefold the response to cold in hypothyroid rats ([8.37–2.45]/[3.62–2.45]; see Table III). The increments in [^{35}S]methionine incorporation after these manipulations were of comparable magnitude to those observed in immunoreactive UCP levels during 48 h of cold exposure (Table I). If the effects of thyroid hormones were due to reduced rates

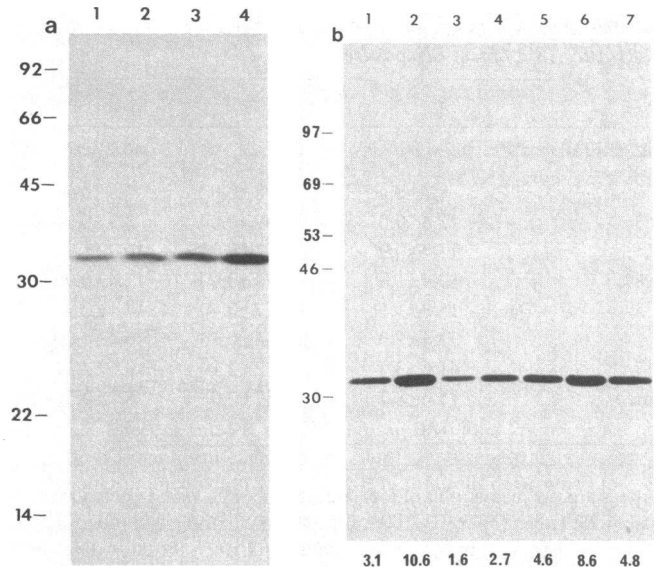

Figure 2. (*a*) SDS PAGE of immunoprecipitates of in vivo [^{35}S]methionine-labeled BAT mitochondrial proteins from Tx rats. Lane *1*, Tx at room temperature; lane *2*, untreated Tx in the cold; lane *3*, T_3-treated Tx in the cold; and lane *4*, as in lane *3* plus one dose of 800 ng T_4/100 g BW at the start of the cold stress. Cold stress lasted for 19 h. [^{35}S]methionine was injected after 16 h at 4 C. T_3 dosage, 150 ng/100 g BW subcutaneously every ~12 h for 5 d. (*b*) SDS PAGE of immunoprecipitated UCP translated in vitro in a rabbit reticulocyte lysate. (Bethesda Research Laboratories) incubated with 1 μg of BAT cytoplasmic mRNA and carrier-free [^{35}S]methionine. Rats were maintained at room temperature or placed in the cold room at 4°C for 19 h; they were grouped and treated as follows; lane *1*, intact rats; lane *2*, intact rats 4°C; lane *3*, Tx rats; lane *4*, Tx rats at 4°C; lane *5*, Tx rats at 4°C and treated with T_3 as for lane *3* in *A*; lane *6*, Tx rats at 4°C treated with T_3 and T_4 as in *A*, lane 4; lane *7*, same as in lane *6* plus IOP 5 mg/100 g BW intraperitoneally 12 h before T_4 administration. Each lane represents the pooled mRNA from four rats. The numbers at the bottom of each lane represent the percent of TCA-insoluble counts that were precipitated with the antibody.

of degradation of UCP, the increments over the untreated animals would have been time dependent, i.e., much larger after 48 h of cold exposure than after 3 h of endogenous labeling. Thus, cold induces a rapid but modest response of UCP to cold in hypothyroid rats and thyroid hormone amplifies the response by increasing the synthetic rate of the protein. As with the immunoreactive levels, the response to cold is just modestly increased by 5-d replacement with T_3 but markedly augmented by one replacement dose of T_4 in a 19-h interval. The T_3 regimen normalized the serum concentration of T_3 and the liver αGPD, as it did in the other experiments, whereas the dose of T_4 did not improve the low level of αGPD nor did it normalize the serum level of T_3 (not shown).

Effect of cold exposure and thyroid hormones on the UCP mRNA levels. To determine whether the thyroid hormone–induced increase in UCP synthesis was pre- or posttranslational, we examined the products of translation of BAT mRNA obtained under the same conditions of the preceeding experiment. Neither the amount of mRNA recovered per gram of tissue nor the recovery of TCA-insoluble ^{35}S counts after translation was significantly affected by the various experimental manipulations. When identical numbers of TCA-precipitable ^{35}S counts were

Table III. In Vivo [^{35}S]Methionine Incorporation into BAT UCP after 19 h of Cold Exposure in Tx Rats Treated with T_3 and/or T_4

Treatment groups	Temperature	UCP	Liver αGPD
	°C	%	ΔOD/min × 10^2
—	25	2.45±0.27	3.4±0.2
—	4	3.62±0.25*	3.5±0.5
T_3	4	4.73±0.39‡	8.9±0.3
T_4	4	8.37±0.39§	4.3±0.6
$T_3 + T_4$	4	8.29±0.34§	8.8±0.2
F		154.6	194.0

Values are the means±SD of three animals. Tx rats were treated with T_3 and/or T_4 as in Fig. 2 *A*. Cold exposure lasted 19 h and [^{35}S]methionine was injected intravenously 3 h before the end of this period. UCP was immunoprecipitated as described under Fig. 2 *A* and expressed as percent of total [^{35}S]methionine-labeled mitochondrial protein. Liver αGPD as inTable I.
One-way analysis of variance: * $P < 0.01$ vs. Tx at 25°C; ‡$P < 0.01$ vs. Tx at 4°C; §$P < 0.01$ vs. Tx + T_3 at 4°C.

immunoprecipitated with the UCP antiserum, the incorporation of [^{35}S]methionine into UCP was found higher in euthyroid than in untreated Tx rats at room temperature (3.1 vs. 1.6% of the TCA-insoluble counts, Fig. 2 *B*). After 19 h of cold exposure there was a 3.5-fold increment in immunoprecipitable counts in intact animals to 10.6% of the TCA-insoluble counts, whereas in the untreated Tx rats this value was 2.7%. T_3 replacement for 5 d as described above improved the response to cold to about one half of that observed in the intact animals, whereas a single injection of 800 ng T_4/100 g BW to Tx rats sufficed to normalize the UCP mRNA response to cold. As above, the blockade of T_3 generation from T_4 with IOP prevented the effect of the latter. Thus, the responses in immunoreactive UCP concentration and in synthetic rate are likely to reflect levels of the corresponding mRNA.

Discussion

UCP is rate limiting for the thermogenic response of BAT (3). It is thanks to this protein that the augmented oxidation of substrates in BAT, largely fatty acids, can result in marked increments in heat production and energy dissipation in overfeeding (1–3). Hypothyroid animals and humans are known to be at risk of hypothermia in cold environments. The protective effect of thyroid hormone has always been considered to result from the stimulation of oxidations in a variety of tissues. We have confirmed that hypothyroid animals cannot maintain their core temperature when exposed to cold and that the abnormality is rapidly corrected by thyroid hormone, but the prevention of the hypothermia correlated with the correction of the UCP levels, not with the correction of liver αGPD.

Because of our earlier demonstration of the activation of BAT 5′D by sympathetic stimulation and the several fold increase in BAT T_3, we suspected that T_3 was more important for BAT than previously recognized (7, 8). On the other hand, the enzyme is rapidly inhibited by high doses of T_4 (22), and the experiments exploring the effect of thyroid hormone on BAT had been done with high doses of T_4 in animals exposed to cold (4, 5). We reasoned that the cold-stimulated deiodinase activity and the magnitude of the T_4 doses, probably resulted in shallow dose–

response curves leading to the concept that thyroid hormones were just permissive. We have shown here that the UCP response is clearly T_3 dose dependent. In addition, we found that the dose of T_3 necessary to normalize UCP is in the hyperthyroid range, which we have documented with the hyperthyroid levels of αGDP activity in the liver. Based in recent studies (9), we calculated that the highest dose of T_3 used in the present experiments maintained the nuclear T_3 receptors ~90% saturated. A calculation of nuclear occupancy in similar experiments (Bianco, A. C., and J. E. Silva, manuscript in preparation) indicates that the UCP response is not linear with nuclear occupancy but of the amplified pattern shown by Oppenheimer et al. for αGPD and malic enzyme in rat liver (23). This, and the finding that the receptors are 70–75% occupied in euthyroid rats at 25°C (9), suggest that the optimal response of this tissue to cold requires a high degree of saturation.

The critical level of BAT T_3 needed for a full response is much more efficiently reached by in situ intracellular generation than from plasma T_3. Only one replacement dose of T_4 can normalize the response of UCP to cold in animals with chronic hypothyroidism, but several days of T_3 replacement were insufficient. The latter, however, normalized both serum T_3 and liver αGPD, documenting the adequacy of the regimen to replace this hormone. Blockade of T_4 to T_3 conversion by IOP prevented the effect of T_4 but not that of T_3, without affecting the plasma levels of either iodothyronine or decreasing the T_4 availability in BAT. This suggests that it is the T_3 generated in BAT, and not plasma T_4 or T_3, the relevant hormone for the UCP response to cold. In previous studies (8), we showed that, after a dose of T_4 identical to that used in the present experiments, 4 h of cold exposure resulted in ~ five times as high locally generated BAT T_3 as that of rats maintained at room temperature. The excess of T_3 was due to the sympathetic activation of BAT 5'D because it was prevented by prazosin, an α_1-antiadrenergic agent that impairs the cold- and the norepinephrine-induced activation of BAT 5'D (7, 8). The present results illustrate the relevance of such activation, for a dose of T_4 insufficient to normalize the serum T_3 concentration or liver αGPD, normalized the UCP response to cold. To obtain the same response with exogenous T_3 requires of doses causing hyperthyroid levels of serum T_3 and liver αGPD.

Acute exposure to low ambient temperature results in a rapid increase of the mRNA levels of UCP (24). T_3 also increases the rate of synthesis of this protein both, at room temperature and in response to cold, which reflects proportional elevations of corresponding mRNA. Whether the effect of thyroid hormone is direct, either by increasing the transcription rate of UCP mRNA or to stabilizing this mRNA, or whether the effect is indirect by amplifying a signal triggered by the sympathetic nervous system, remains to be demonstrated. Whatever the mechanism involved, this is another situation where thyroid hormone amplifies the response to a primary stimulus, in this case the sympathetic nervous system, as it does with the response of lipogenic enzymes to carbohydrates (25), and in that constitutes an excellent model to study thyroid hormone action at molecular level. Of course, the use of UCP as a model of thyroid hormone action at cellular level mandates to demonstrate that the UCP responses observed here are direct e.g., not mediated through an effect on the adrenergic pathway, which will probably need the development of an in vitro system. Because BAT function in hibernating species is to warm the hypothermic body of these animals at the end of hibernation (2, 3), the possibility that hypothermia itself impaired the raise of UCP in the present experiments is remote, but also must be excluded.

Two corollaries emerge from the results presented here. First, that BAT 5'D is a key element in the function of BAT and hence a variable that may affect the physiological responses of this tissue. This conclusion is in agreement with the observation that ob/ob mice die of hypothermia when exposed to cold, for in these animals the BAT 5'D is not activated by the cold as in lean controls (26). Secondly, that at euthyroid levels of T_4 and T_3, the thermogenic effect of T_3, evidenced in its ability to prevent hypothermia at low ambient temperature, results largely from its action on BAT and not from its various and widespread metabolic effects in other tissues. The present data suggest that the stimulation of UCP synthesis is one of the mechanisms by which thyroid hormone exerts this physiological effect, but the possibility that T_3 affects other aspects of BAT metabolism leading to the same results, remains open. Because the calorigenic BAT response may be limited by the saturation of the receptors, one would expect that the increased thermogenesis observed in hyperthyroidism results largely from the direct action of thyroid hormones in other tissues, where an increase in respiration has been well documented (27, 28).

Acknowledgments

We are grateful of Dr. Jean Himms-Hagen for the gift of antiserum against the uncoupling protein which was helpful to identify this protein during the purification. We also appreciate the secretarial help of Janice Bridges.

Dr. Bianco is a recipient of a Fellowship from the Fundacao de Amparo a Pesquisa do Estado de Sao Paulo, Brazil.

References

1. Rothwell, N. J., and M. J. Stock. 1983. Diet-induced thermogenesis. *In* Mammalian Thermogenesis. L. Girardier and M. J. Stock, editors. Chapman & Hall, London. 208–233.

2. Himms-Hagen, J. 1985. Brown adipose tissue metabolism and thermogenesis. *Annu. Rev. Nutr.* 5:69–94.

3. Cannon, B., and J. Nedergaard. 1985. The biochemistry of an inefficient tissue: brown adipose tissue. *Essays Biochem.* 20:110–163.

4. Mory, G., D. Ricquier, P. Pesquies, and P. Hemon. 1981. Effects of hypothyroidism on the brown adipose tissue of adult rats: comparison with the effects of adaptation to cold. *J. Endocrinol.* 91:515–524.

5. Triandafillou, J., C. Gwillian, and J. Himms-Hagen. 1982. Role of thyroid hormone in cold-induced changes in rat brown adipose tissue mitochondria. *Can. J. Biochem.* 60:53–537.

6. Leonard, J. L., S. A. Mellen, and P. R. Larsen. 1983. Thyroxine 5'-deiodinase activity in brown adipose tissue. *Endocrinology.* 112:1153–1155.

7. Silva, J. E., and P. R. Larsen. 1983. Adrenergic activation of triiodothyronine production in brown adipose tissue. *Nature (Lond.).* 305: 712–713.

8. Silva, J. E., and P. R. Larsen. 1985. Potential of brown adipose tissue Type II thyroxine 5'-deiodinase as a local and systemic source of triiodothyronine in rats. *J. Clin. Invest.* 76:2296–2305.

9. Bianco, A. C., and J. E. Silva. 1987. Nuclear T3 in brown adipose tissue. Receptor occupancy and sources of T3 as determined by in vivo techniques. *Endocrinology.* In press.

10. Lin, C. S., and M. Klingenberg. 1980. Isolation of the uncoupling protein from brown adipose tissue mitochondria. FEBS *(Fed. Eur. Biochem. Soc.)* Letters 113:299–303.

11. Klingenberg, M., H. Aquila, and P. Riccio. 1979. Isolation of functional membrane proteins related to or identical with the ADP, ATP carrier of mitochondria. *Methods Enzymol.* 56:407–418.

12. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature (Lond.).* 227:680–685.

13. Langone, J. J. 1980. 125-I labeled protein A: reactivity with IgG and use as a traceer in radioimmunoassay. *Methods Enzymol.* 70:356–375.

14. Comstock, J. P., L. Chan, and A. R. Means. 1986. Messenger RNA translation and protein synthesis. *In* Laboratory Methods Manual for Hormone Action and Molecular Endocrinology. W. T. Schrader and B. W. O'Malley, editors. *Houston Biological Association, Houston.* 1–15.

15. Sajdel-Sulkowska, E., J. F. Coughlin, and C. A. Marotta. 1983. In vitro synthesis of polypeptides of moderately large size by poly(A)-containing messenger RNA from postmortem human brain and mouse brain. *J. Neurochem.* 40:670–680.

16. Wreschner, D., and M. Herzberg. 1984. A new blotting medium for the simple isolation and identification of highly resolved messenger RNA. *Nucleic Acids Res.* 12:1349–1359.

17. Pelham, H. R. B., and R. J. Jackson. 1976. An efficient mRNA-dependent translation system from reticulocyte lysates. *Eur. J. Biochem.* 67:247–256.

18. Lee, Y. P., and H. Lardy. 1965. Influence of thyroid hormones on L-α-glycerophosphate dehydrogenases and other dehydrogenases in various organs of the rat. *J. Biol. Chem.* 240:1427–1436.

19. Gardner, R. S. 1974. A sensitive colorimetric assay for mitochondrial α-glycerophosphate dehydrogenase. *Anal. Biochem.* 59:272–276.

20. Silva J. E., J. L. Leonard, F. R. Crantz, and P. R. Larsen. 1982. Evidence for two tissue-specific pathways for in vivo thyroxine 5'deiodination in the rat. *J. Clin. Invest.* 69:1176–1184.

21. Larsen, P. R., and R. D. Frumess. 1977. Comparison of the biological effects of thyroxine and triiodothyronine in the rat. *Endocrinology.* 100:98–988.

22. Silva, J. E., and P. R. Larsen. 1986. Interrelationships among thyroxine, growth hormone, and the sympathetic nervous system in the regulation of 5'-iodothyronine deiodinase in rat brown adipose tissue. *J. Clin. Invest.* 77:1214–1223.

23. Oppenheimer, J. H., P. Coulombe, H. L. Schwartz, and N. W. Gutfeld. 1978. Nonlinear (amplified) relationship between nuclear occupancy by triiodothyronine and the appearance rate of hepatic α-glycerophosphate dehydrogenase and malic enzyme in the rat. *J. Clin. Invest.* 61:987–997.

24. Jacobsson, A., U. Stadler, M. A. Glotzer, and L. P. Kozak. 1985. Mitochondrial uncoupling protein from mouse brown fat. *J. Biol. Chem.* 260:16250–16254.

25. Mariash, C. N., and J. H. Oppenheimer. 1983. Thyroid hormone-carbohydrate interaction. *In* Molecular Basis of Thyroid Hormone Action. J. H. Oppenheimer and H. H. Samuels, editors. Academic Press, Inc., New York. 265–292.

26. Kates, A. L., and J. Himms-Hagen. 1985. Defective cold-induced stimulation of thyroxine 5'-deiodinase in brown adipose tissue of the genetically obese (ob/ob) mouse. *Biochem. Biophys. Res. Commun.* 130: 188–193.

27. Barker S. B., and H. M. Klitgaard. 1952. Metabolism of tissues excised from thyroxine-injected rats. *J. Physiol.* 170:81–86.

28. Bachman K., A. Arieli, A. G. Burger, and A. E. Chinet. 1985. Triiodothyronine (T3)-induced thermogenesis: altered T3-efficiency in tissues from fed, starved, and refed hypothyroid rats. *Endocrinology.* 117:1084–1089.