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# Thermogenic effect of triiodothyroacetic acid at low doses in rat adipose tissue without adverse side effects in the thyroid axis

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**Medina-Gomez G, Calvo RM, Obregon M-J.** Thermogenic effect of triiodothyroacetic acid at low doses in rat adipose tissue without adverse side effects in the thyroid axis. *Am J Physiol Endocrinol Metab* 294: E688–E697, 2008. First published February 19, 2008; doi:10.1152/ajpendo.00417.2007.—Triiodothyroacetic acid (TRIAC) is a physiological product of triiodothyronine ($T_3$) metabolism, with high affinity for $T_3$ nuclear receptors. Its interest stems from its potential thermogenic effects. Thus this work aimed *1*) to clarify these thermogenic effects mediated by TRIAC vs. $T_3$ in vivo and *2*) to determine whether they occurred predominantly in adipose tissues. To examine this, control rats were infused with equimolar $T_3$ or TRIAC doses (0.8 or 4 nmol·100 g body $wt^{-1}$·$day^{-1}$) or exposed for 48 h to cold. Both $T_3$ doses and only the highest TRIAC dose inhibited plasma and pituitary thyroid-stimulating hormone (TSH) and thyroxine ($T_4$) in plasma and tissues. Interestingly, the lower TRIAC dose marginally inhibited plasma $T_4$. $T_3$ infusion increased plasma and tissue $T_3$ in a tissue-specific manner. The highest TRIAC dose increased TRIAC concentrations in plasma and tissues, decreasing plasma $T_3$. TRIAC concentrations in tissues were $<10\%$ those of $T_3$. Under cold exposure or high $T_3$ doses, TRIAC increased only in white adipose tissue (WAT). Remarkably, only the lower TRIAC dose activated thermogenesis, inducing ectopic uncoupling protein (UCP)-1 expression in WAT and maximal increases in UCP-1, UCP-2, and lipoprotein lipase (LPL) expression in brown adipose tissue (BAT), inhibiting UCP-2 in muscle and LPL in WAT. TRIAC, $T_3$, and cold exposure inhibited leptin secretion and mRNA in WAT. In summary, TRIAC, at low doses, induces thermogenic effects in adipose tissues without concomitant inhibition of TSH or hypothyroxinemia, suggesting a specific role regulating energy balance. This selective effect of TRIAC in adipose tissues might be considered a potential tool to increase energy metabolism.

thermogenesis; leptin; lipoprotein lipase; deiodinases

THE MAIN PATHWAYS of triiodothyronine ($T_3$) metabolism are deiodination, conjugation with glucuronides and sulfates, and modification of the alanine chain producing acetic and propionic metabolites. Triiodothyroacetic acid (TRIAC, also known as Tiratricol) is a physiological product of $T_3$ metabolism, derived by deamination and oxidative decarboxylation of the alanine chain (55). In humans, TRIAC production by the liver and other tissues accounts for $\sim$14% of $T_3$ metabolism (17). This production is increased under certain physiological conditions, such as fasting (29). Serum TRIAC concentrations in humans are very low (42–140 pM) (17, 35, 38). The free fraction in serum is lower than that of $T_3$, due to higher binding to plasma proteins, especially to transthyretin (18, 35). TRIAC has a faster metabolic clearance rate and shorter half-life than $T_3$ in humans (35), due to its rapid hepatic metabolism, via the formation of sulfates and glucuronides, especially in humans (36, 51).

TRIAC has a higher affinity than $T_3$ for thyroid hormone nuclear receptors (TR), especially the $\beta_1$-isoform of the nuclear TR (TR-$\beta_1$), in various cell types (52) and also in rat brown adipocytes (20). In pituitary cells "in vitro," TRIAC elicits a similar response to $T_3$, inhibiting thyroid-stimulating hormone (TSH) secretion (16). In fact, TRIAC has been used to suppress TSH secretion in patients with thyroid hormone resistance, inappropriate TSH secretion, or thyroid carcinoma (2, 23, 60) because it exerts fewer effects on cardiac function than $T_3$. However, due to its rapid metabolism in humans, high daily doses are required (0.4 to 1–2 mg TRIAC), 200 times higher than physiological doses of $T_3$. At these high doses, TRIAC induces persistent hypothyroidism due to TSH suppression. Other thyromimetic actions of TRIAC include decreases in cholesterol or lipids and an increase in the expression of several $T_3$-dependent genes [sex hormone-binding globulin (SHBG), Spot 14 (S14), type I 5′-deiodinase (D1)] (9, 22, 26, 53, 54) in liver, a tissue with predominant TR-$\beta_1$ expression.

A specific role, different from that of $T_3$, has not been ascribed to TRIAC. Previous attempts to demonstrate TRIAC effects promoting oxygen consumption or energy expenditure have been inconclusive. Whereas some early experiments reported that TRIAC increased oxygen consumption (47) and suggested a possible role regulating energy expenditure (13), other studies failed to do so (3, 27), despite the fact of evidence of thyromimetic actions on lipids (9, 23, 53). Brown adipose tissue (BAT) is specialized in the production of heat in facultative thermogenesis. This function is mediated by uncoupling protein (UCP)-1 (39). Cold exposure and cafeteria diets are typical experimental paradigms used to stimulate BAT activity and UCP-1 expression via the release of norepinephrine (NE) from sympathetic nerve endings. BAT activity is an important determinant of energy balance (61). Thyroid hormones are essential for basal and facultative thermogenesis, in particular for the full expression of UCP-1 (5, 58). UCP-1 expression and its response to cold are low in hypothyroid adult rats, fetuses, and newborns (6, 43). Thyroid hormone administration restores UCP-1 mRNA levels and its response to cold (6, 41). $T_3$ increases the transcription rate, stabilizes UCP-1 mRNA transcripts, and increases the effect of NE (4, 5, 48). $T_3$ concentrations in BAT are elevated, especially under cold exposure and during fetal life (44, 57). $T_3$ in BAT is produced by type II 5′-deiodinase (D2), which is activated in response to cold (56).

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This $T_3$ production is required for the high saturation of nuclear $T_3$ receptors in response to cold exposure and for optimal thermogenic function (57).

Our previous studies using brown adipocytes have shown that TRIAC at very low concentrations (0.2 nM) had more thermogenic effect than $T_3$ (34), increasing the adrenergic stimulation of both UCP-1 mRNA and D2 activities. Therefore we hypothesized that TRIAC could also be a thermogenic agent in vivo that might increase energy expenditure and regulate $T_3$ production (by increasing D2) in BAT. The objective of the present study was to characterize the specific effects of TRIAC in vivo and to compare them with those of $T_3$, especially in adipose tissue. Of particular interest was to define the therapeutic potential of TRIAC metabolic effects at low doses. Our results show that TRIAC at low doses is able to induce ectopic UCP-1 in white adipose tissue (WAT), that TRIAC is effective in leading to multiple changes in thermogenic and lipid-related gene expression in adipose tissues, and that these changes are not found in all the tissues with predominant TR-β1 expression (i.e., liver).

## MATERIALS AND METHODS

*Materials.* Thyroxine ($T_4$), $T_3$, 3,5-diiodothyronine, DTT, 6-*n*-propyl-2-thiouracil (PTU), MOPS, and agarose were obtained from Sigma (St. Louis, MO). TRIAC, diiodothyroacetic acid (DIAC), reverse $T_3$ ($rT_3$) and 3′,3-diiodothyronine were obtained from Henning Berlin (Berlin, Germany). $^{125}$I, $^{131}$I, and [α-$^{32}$P]dCTP were obtained from Amersham International (Aylesbury, UK). The oligolabeling system was from Pharmacia (Uppsala, Sweden). Nytran membranes were from Schleicher & Schuell (Dassel, Germany). All other chemicals were molecular biology grade.

*Experimental design.* Female Sprague-Dawley rats were used. They were housed under humane conditions, under veterinary control, according to the European Community guidelines and after approval by the Ethics Committee of our institution. The rats were maintained at 22°C with 12-h periods of light and darkness and were fed a stock pellet diet (Sandermus; Sanders, Barcelona, Spain). The rats (243 ± 3 g body wt, $n$ = 5/group) were implanted with osmotic minipumps (2ML 2; Alza, Palo Alto, CA) under the dorsal skin that delivered two equimolar doses of $T_3$ or TRIAC (0.8 or 4 nmol·100 g body wt$^{-1}$·day$^{-1}$, which correspond to 0.52 and 2.6 μg $T_3$ and 0.5 and 2.49 μg TRIAC·100 g body wt$^{-1}$·day$^{-1}$). The doses were chosen following previous experiments done infusing increasing doses of $T_3$ (14, 15) on the basis of the $T_3$ concentrations found in several tissues using low and high $T_3$ doses. After 12 days of continuous infusion, the animals were killed. After being bled, several tissues were obtained and quickly frozen on dry ice. Skeletal muscle was obtained from the hind leg and WAT from the abdominal depots, excluding perirenal depots. One group of control rats was infused with placebo, and another group was maintained in individual cages in a cold room at 8°C for 48 h. The $T_3$ and TRIAC doses for infusion were dissolved using 0.05 N NaOH and were diluted in saline + 2% BSA.

*RIAs of $T_4$, $T_3$, and TRIAC in plasma and tissues.* Thyroid hormone concentrations were determined by RIA after extraction and purification of plasma and tissues as previously described (37) by using methanol-chloroform extraction, back extraction into an aqueous phase, and purification of plasma and tissue extracts on DOWEX 1X2 columns. This procedure retains TRIAC in the DOWEX, avoiding any interference of TRIAC in the $T_3$ RIA. Thyroid hormones were determined in the purified extracts by using sensitive RIAs (37). The cross-reactivity and sensitivity of the RIAs were described (50). Samples were processed in duplicate at two dilutions. The final results were calculated by using individual recovery data obtained by the addition of tracer amounts of [$^{131}$I]$T_4$ and [$^{125}$I]$T_3$ to the initial homogenates.

TRIAC concentrations were determined in extracts from plasma and tissues by specific RIA, using a specific TRIAC antibody (kindly provided by Dr. A. Burger). Plasma and tissue homogenates were used, and 2,000 cpm of [$^{125}$I]TRIAC were added to each tube to calculate individual recoveries, extracted with methanol, and filtered through Microsep microconcentrators (3K filters; Filtron Technology, Pall, NY). The purified extracts were evaporated to dryness and were reconstituted with RIA buffer. The standard curve was done in buffer (0.04 M phosphate, pH = 8, 0.2% BSA, and 0.6 mM merthiolate) and ranged from 0.9 to 250 pg TRIAC/tube, with a detection limit of 0.9 pg TRIAC/tube. Cross-reactivity with $T_3$ was 0.16%.

*TSH, growth hormone, and leptin RIAs.* TSH was determined in 50 μl of plasma by using the reactives for rat TSH RIA, kindly supplied by the National Institutes of Health (Bethesda, MD), and were made available through the Rat Pituitary Agency of the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK). Concentrations are expressed in weight equivalents of the NIDDK-rTSH-RP-3 reference preparation. [$^{125}$I]TSH was labeled by using lactoperoxidase. Hyperthyroid rat serum was used to set up the standard curve to improve the sensitivity of the TSH RIA (0.06 ng/ml plasma). TSH and growth hormone (GH) were also determined in pituitary glands by using standard curves in buffer. One-fiftieth of the pituitary was further diluted 1:100 for TSH and 1:10.000 for GH. Leptin concentrations were measured by using a sensitive and specific rat leptin RIA kit from Linco Research (St. Charles, MO). The intra- and intercoefficients of variation for this assay were 4.0% and 11.2%, respectively.

*Radioactive products.* High-specific-activity [$^{131}$I]$T_4$, [$^{125}$I]$T_4$, [$^{125}$I]$T_3$, [$^{125}$I]$rT_3$, and [$^{125}$I]TRIAC (3,000 μCi/μg) were synthesized in our laboratory from the substrate with a lower degree of iodination and were used for the highly sensitive $T_4$, $T_3$, and TRIAC RIAs; as recovery tracers for extraction; and as substrates for D1 and D2.

*Iodothyronine 5′-deiodinase (D1 and D2) activities.* Before each assay, [$^{125}$I]$rT_3$ or [$^{125}$I]$T_4$ was purified by paper electrophoresis to separate the iodide. Iodothyronine 5′-deiodinase activities were assayed as described (44, 50). The experimental conditions for D1 in liver and kidney were 400 nM $rT_3$ ([$^{125}$I]$rT_3$) ± 1 mM PTU and 2 mM DTT for 10 min (30 μg protein) and for D1 in heart and pituitary were 2 nM $rT_3$ ([$^{125}$I]$rT_3$) ± 1 mM PTU and 20 mM DTT for 1 h. For D2 activity in BAT and pituitary, conditions were 2 nM $T_4$ ([$^{125}$I]$T_4$) + 1 μM $T_3$ and 20 mM DTT in the presence of 1 mM PTU for 1 h (150–200 μg protein). All incubations were done at 37°C. The $^{125}$I released was separated by ion-exchange chromatography on DOWEX 50W-X2 columns equilibrated in 10% acetic acid. The protein content was determined by the method of Lowry et al. (30), after precipitation of the homogenates with 10% TCA to avoid interferences from DTT in the colorimetric reaction.

*RNA preparation and Northern blot analysis.* Total RNA was extracted by using Trizol (GIBCO-BRL Life Technologies, Grand Island, NY). Total RNA (15 μg) was denatured and electrophoresed on a 2.2 M formaldehyde-1% agarose gel in 1× MOPS buffer and was transferred to nylon membranes. Several genes were analyzed: UCP-1 (8), UCP-2 and leptin (33), UCP-3, lipoprotein lipase (LPL) and α- and β-myosin heavy chain (MHC), and hyperpolarization-activated cyclic nucleotide-gated channel 2 (HCN2) (46). The cDNAs were used as templates for [α-$^{32}$P]dCTP-labeled probes by using random primers (>$10^8$ cpm/μg DNA). Filters were hybridized and washed under standard conditions. Alternatively for leptin and UCP-3, ULTRAHyb (Ambion, Huntingdon, UK) was used. Autoradiograms were obtained from the filters and were quantified by laser computer-assisted densitometry (Molecular Dynamics, Sunnyvale, CA) or NIH Image software. The membranes were routinely dyed with methylene blue to visualize the ribosomal RNAs, and differences between lanes were used to correct the results obtained (cyclophilin expression was very low in some tissues such as muscle and WAT). Results are expressed as means ± SE of two to four determinations.

*Statistical analysis.* Mean values ± SE are given. When not visible in the figures, SE was smaller than the size of the symbols. One-way analysis of variance was applied after ensuring homogeneity of variance by Bartlett's test. Statistically significant differences between mean values of different groups were then identified by the least significant difference method. All calculations were performed as described by Snedecor and Cochran (59).

## RESULTS

*Low doses of TRIAC inhibit less plasma $T_4$ and TSH than $T_3$.* As expected, $T_3$ and TRIAC inhibited plasma $T_4$. However, at equimolar doses the infusion of $T_3$ had more effect than TRIAC at inhibiting $T_4$ (<10% of control values for both $T_3$ doses; Fig. 1), whereas the effect of TRIAC was smaller (70% and 40% of control values for the low and high TRIAC doses).

At the lowest dose, TRIAC did not affect plasma TSH, whereas both doses of $T_3$ and the highest dose of TRIAC markedly reduced TSH levels. Pituitary TSH content mimicked the pattern observed in plasma TSH, suggesting that changes in plasma TSH were secondary to changes in pituitary TSH. The low TSH stimulation led to low $T_4$ thyroidal secretion. Pituitary GH did not change under $T_3$ or TRIAC infusion.

Furthermore, the infusion of $T_3$ and TRIAC to control rats increased their respective plasma concentrations in proportion to the doses infused, and the increases were higher for TRIAC than for $T_3$ (1.5- and 3.6-fold increases in plasma $T_3$ for both $T_3$ doses and 4.5- and 18-fold increases in plasma TRIAC for both TRIAC doses). This could be related to the higher binding of TRIAC to plasma proteins. $T_3$ concentrations in plasma decreased by 50% by infusion of the highest TRIAC dose, whereas none of the $T_3$ doses increased plasma TRIAC concentrations. Cold exposure for 48 h did not alter plasma $T_4$ or TRIAC but increased plasma $T_3$ and TSH, with no changes in pituitary TSH.

*Low doses of TRIAC do not affect $T_4$ and $T_3$ concentrations in rat tissues.* $T_4$ and $T_3$ concentrations were measured in BAT, WAT, heart, skeletal muscle, liver, and kidney (Fig. 2). $T_4$ concentrations (*top* panels) were decreased in all tissues by both $T_3$ doses; however, the decreases observed were less pronounced than the changes seen in plasma $T_4$. Conversely, the lowest TRIAC dose did not inhibit $T_4$ in tissues, whereas the highest TRIAC dose resulted in a smaller inhibitory effect (~50% of control, except for BAT $T_4$).

With respect to $T_3$ concentrations, they changed heterogeneously among the tissues studied (Fig. 2, *bottom* panels). For instance, $T_3$ infusion led to minimal increases in BAT $T_3$ levels, suggesting that circulating $T_3$ is probably less relevant than locally produced $T_3$ in BAT. In WAT, only the highest dose of $T_3$ induced a threefold increase in WAT $T_3$. In heart and muscle, the infusion of both $T_3$ doses resulted in similar increases (two- and fourfold increases). Finally, in liver and kidney only the high $T_3$ dose led to clear increases (four- and sixfold, respectively). Cold exposure increased BAT $T_3$ and $T_4$, $T_3$ in heart and muscle, and liver $T_4$. Of interest, only the highest dose of TRIAC reduced $T_3$ by 50% in heart, liver, and kidney, parallel to levels of $T_3$ in plasma. Therefore the effects observed in the TRIAC-infused rats could be attributed to TRIAC, because $T_3$ did not increase after TRIAC infusion.

Together, these data indicate that low doses of TRIAC do not decrease $T_4$ concentrations in tissues, similarly to what happened in plasma.

*Concentrations of TRIAC in different rat tissues.* TRIAC concentrations were ~10–20% of $T_3$ concentrations in each tissue studied. The highest concentration of TRIAC was found in BAT, followed by heart > WAT > muscle > kidney > liver (range: 0.07–0.4 ng/g tissue, 0.12–0.64 nM).

Infusion of TRIAC at the highest dose increased TRIAC concentrations in BAT (Fig. 3). TRIAC concentrations in WAT only reached 50% of BAT (Fig. 3) and increased less with 4 nmol of TRIAC than in BAT. Interestingly, the highest $T_3$ dose and cold exposure increased TRIAC concentrations by twofold in WAT, suggesting $T_3$ as the main source of TRIAC in both situations, a finding only observed in WAT. In heart, TRIAC concentrations, although higher, followed a similar pattern to those in BAT. The lowest TRIAC concentrations

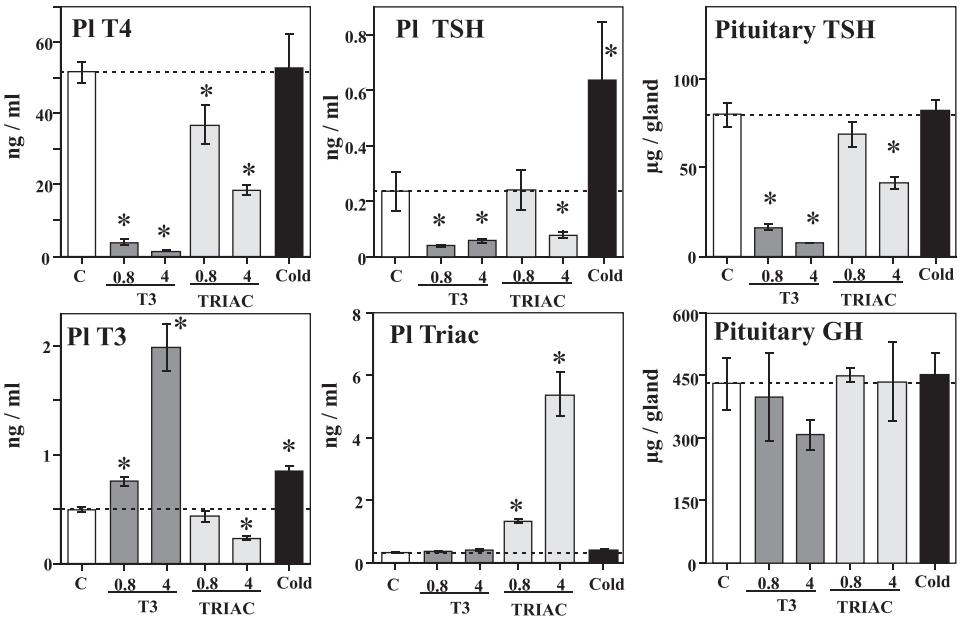


Fig. 1. Plasma thyroid-stimulating hormone (TSH), thyroxine ($T_4$), triiodothyroxine ($T_3$), and triiodothyroacetic acid (TRIAC) concentrations and TSH and growth hormone (GH) pituitary contents. Control rats (C) were infused with 0.8 or 4 nmol of TRIAC or $T_3 \cdot 100$ g body $wt^{-1} \cdot day^{-1}$ for 12 days or were exposed to cold for 48 h (Cold). Plasma $T_4$, $T_3$, TSH, and TRIAC concentrations and TSH and GH pituitary contents are shown (means ± SE). *$P < 0.05$ vs C.

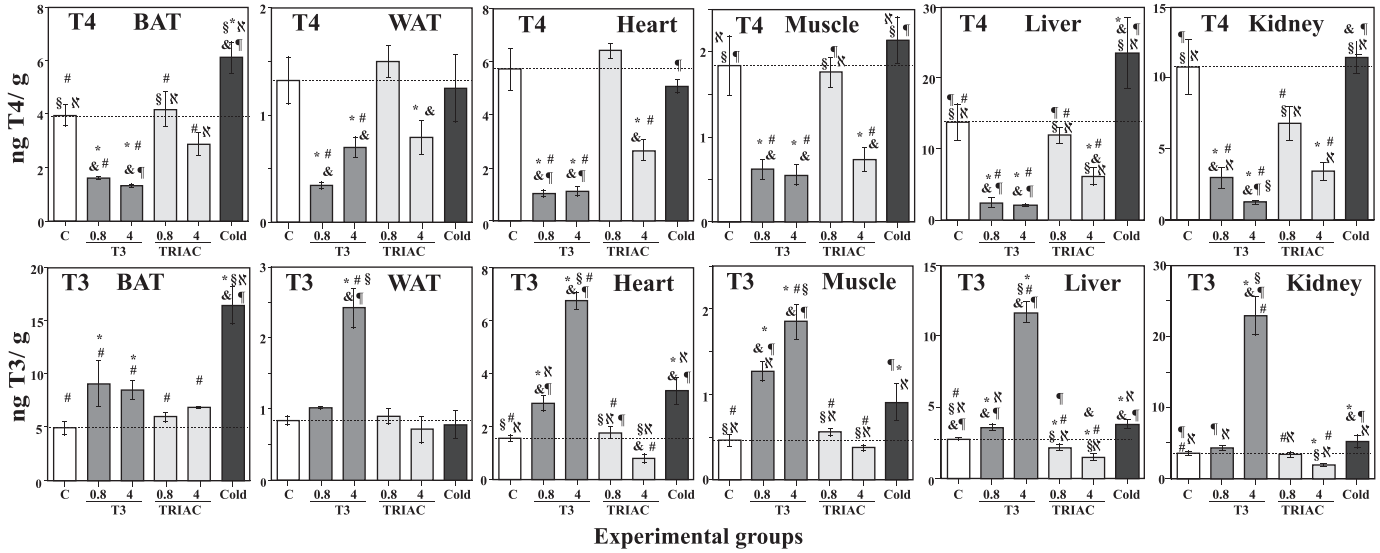


Fig. 2. $T_4$ and $T_3$ concentrations found in brown adipose tissue (BAT), white adipose tissue (WAT), heart, skeletal muscle, liver, and kidney. $T_4$ and $T_3$ were determined by RIAs in purified tissue extracts. Experimental groups are same as described in Fig. 1 (means ± SE). *$P < 0.05$ vs. C, #$P < 0.05$ vs. Cold, &$P < 0.05$ vs. 0.8 nmol TRIAC, ¶$P < 0.05$ vs. 4 nmol TRIAC, §$P < 0.05$ vs. 0.8 nmol $T_3$, and ℵ$P < 0.05$ vs. 4 nmol $T_3$.

were found in muscle, kidney, and liver. Finally, the largest increases of TRIAC were found in liver (12- and 45-fold for both TRIAC doses). In kidney, the increases were similar to plasma and heart.

The tissue-to-plasma ratios for TRIAC were ~1 in BAT and heart and lower in the rest of the tissues (0.3–0.6) and were always lower than $T_3$ tissue-to-plasma ratios (>1 in all tissues, 10–20 in BAT).

*Induction of ectopic UCP-1 in WAT by the lowest dose of TRIAC.* We hypothesized that TRIAC could be a thermogenic agent, so we investigated whether TRIAC could upregulate the expression of UCP-1 in BAT. As expected, cold exposure increased UCP-1 mRNA in BAT (Fig. 4). The lowest TRIAC dose induced UCP-1 expression in BAT more effectively than either $T_3$ dose or the highest TRIAC dose. Interestingly, and unexpectedly, the lowest TRIAC dose (0.8 nmol) induced ectopic expression of UCP-1 in abdominal WAT (3 different rats). This effect was not observed with either the highest TRIAC dose, both doses of $T_3$, or even under cold exposure. These effects on UCP-1 expression were associated with TRIAC concentrations in BAT of ~10% those of $T_3$ (1 nM TRIAC vs. 10 nM $T_3$), and only 0.3 nM TRIAC was found in WAT after the infusion of the lowest TRIAC dose (Figs. 2 and 3). Therefore, these actions of TRIAC in WAT were exerted at very low

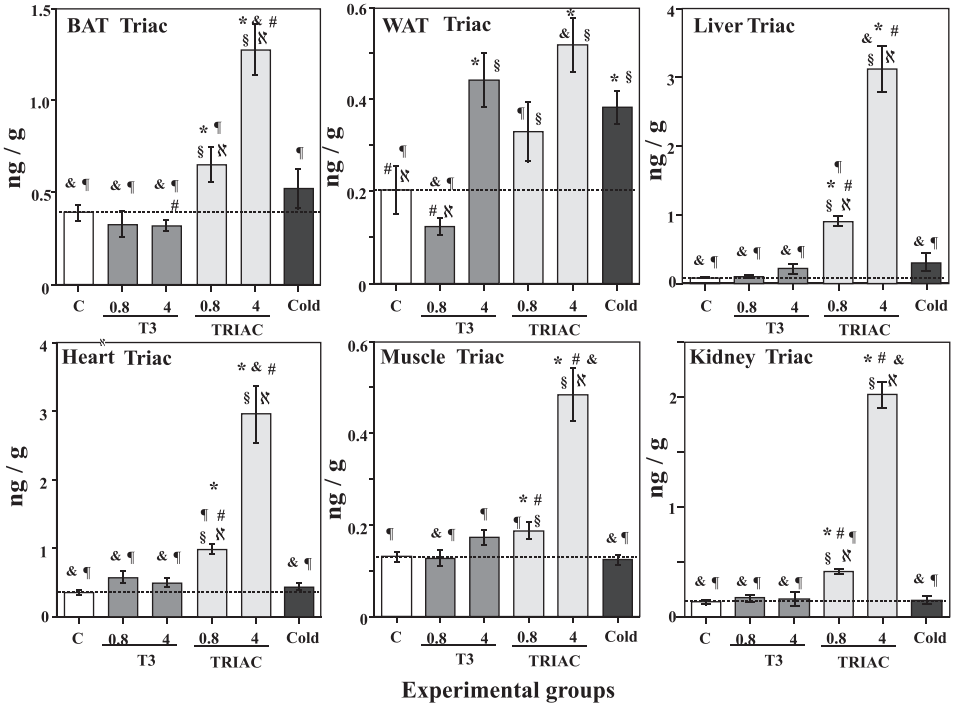


Fig. 3. TRIAC concentrations found in BAT, WAT, heart, skeletal muscle, liver, and kidney. TRIAC was determined by RIA in purified tissue extracts. Experimental groups are same as described in Fig. 1 (means ± SE). *$P < 0.05$ vs. C, #$P < 0.05$ vs. Cold, &$P < 0.05$ vs. 0.8 nmol TRIAC, ¶$P < 0.05$ vs. 4 nmol TRIAC, §$P < 0.05$ vs. 0.8 nmol $T_3$, and ℵ$P < 0.05$ vs. 4 nmol $T_3$.

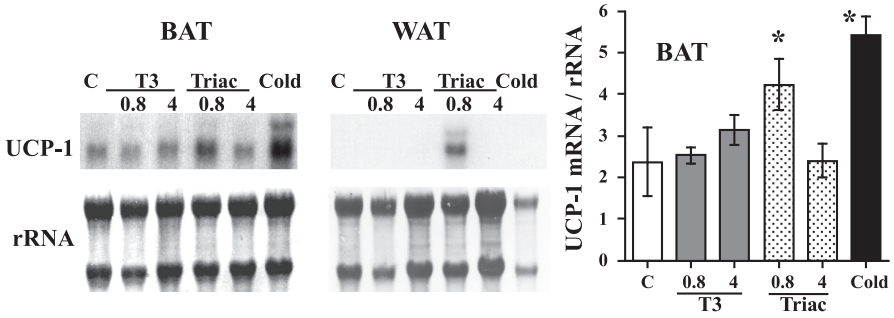


Fig. 4. Uncoupling protein (UCP)-1 mRNA expression in BAT and WAT. Total RNA was extracted from BAT and WAT of rats from experimental groups described in Fig. 1. Hybridization with UCP-1, rRNA staining, and UCP-1/rRNA ratio are shown (means ± SE, $n = 2$–3). Results for WAT were repeated with 3 different rats/group. *$P < 0.05$ vs. C.

concentrations compared with $T_3$ and with no change in $T_3$ concentrations. D2 expression was also measured in WAT, and it was not induced by the low TRIAC dose (results not shown).

The expression of UCP-2 and UCP-3 in different tissues was also examined. In BAT, UCP-2 mRNA showed the greatest increase in response to the lower dose of TRIAC, cold exposure, and both doses of $T_3$. The highest TRIAC dose had no effect (Fig. 5). In WAT, UCP-2 mRNA was only increased by the lowest dose of $T_3$. In muscle, $T_3$ and TRIAC had an inhibitory effect on UCP-2 mRNA, with the lowest TRIAC dose, the highest $T_3$ dose, and cold exposure being more effective. In heart, UCP-2 mRNA expression was higher than in other tissues, and both doses of $T_3$ and TRIAC induced UCP-2 with similar effect. Cold exposure induced UCP-2 mRNA fourfold. Therefore the regulation of UCP-2 is tissue specific, and the lowest TRIAC dose has a similar effect to the highest $T_3$ dose only in BAT and muscle.

The expression of UCP-3 mRNA was only detectable in BAT and muscle. The highest $T_3$ dose and cold exposure had a maximal effect in BAT and muscle UCP-3. There was no effect using both TRIAC doses and the lowest $T_3$ dose, in contrast to the findings for UCP-1 and UCP-2. Some induction of UCP-3 was observed in WAT by using the highest $T_3$ dose and the lowest TRIAC dose (not shown), but UCP-3 could not be quantified.

*Expression of LPL in BAT, WAT, and heart.* LPL recruits the lipids necessary for mitochondrial combustion in BAT, and

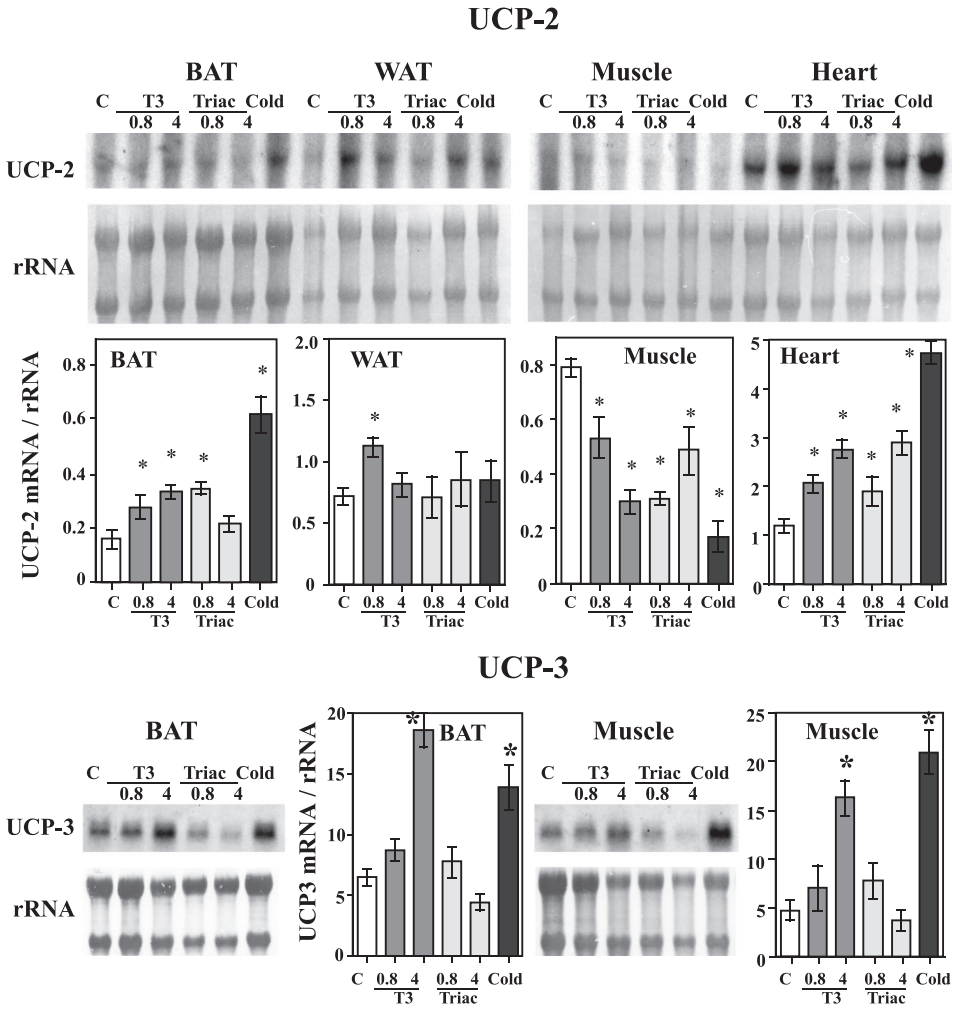


Fig. 5. UCP-2 and UCP-3 mRNA expression in BAT, WAT, heart, and muscle. Total RNA was extracted from BAT, WAT, heart, and skeletal muscle of rats from experimental groups described in Fig. 1. Hybridization with UCP-2 and UCP-3, rRNA staining, and UCP-2 or UCP-3/rRNA ratio are shown (means ± SE, $n = 2$). *$P < 0.05$ vs. C.

cold exposure increases LPL activity in BAT (10). In BAT, LPL mRNA was induced by the lowest $T_3$ and TRIAC doses (Fig. 6) and was inhibited by the highest $T_3$ dose, similarly to our previous findings in cultured brown adipocytes (34). An inhibitory pattern in LPL expression was observed in WAT, with maximum inhibition observed following the highest $T_3$ dose, the lowest TRIAC dose, and after cold exposure. These findings indicate new aspects in the dose-dependent responses of LPL in BAT and WAT. Again, the lowest TRIAC dose and the highest $T_3$ dose had a similar effect, pointing to a higher effect of TRIAC. LPL has been studied in heart previously (7, 40). TRIAC had no effect on LPL in heart, and only cold exposure and the highest $T_3$ dose inhibited LPL in heart, similar to the findings in WAT.

*Leptin secretion and mRNA levels are inhibited by TRIAC and $T_3$.* In our previous studies (33), we showed that leptin secretion and mRNA were inhibited by $T_3$ and TRIAC in white and brown adipocytes. In this study, we found that plasma leptin was inhibited by $T_3$, TRIAC, and cold exposure (Fig. 7) and that the highest $T_3$ dose had a maximal effect. Leptin mRNA was also inhibited in WAT, but the maximal effect was observed with the highest TRIAC dose and cold exposure. Again, the highest $T_3$ dose and the lowest TRIAC dose had a similar effect. In BAT, leptin mRNA expression was too low to be quantified (not shown).

*Effect of TRIAC and $T_3$ on D1 and D2.* The deiodinases are the main source of $T_3$ in tissues and are regulated by $T_3$. We examined the responses of D1 and D2 in tissues with different levels of TR-$\alpha_1$ or TR-$\beta_1$ isoforms. Liver and kidney D1 activities increased with the high doses of $T_3$ and TRIAC (Fig. 8). Maximal increases were obtained with the highest $T_3$ dose, TRIAC being less effective in the induction of D1 activity. In heart, only the highest $T_3$ dose increased D1 activity, whereas none of the TRIAC doses had an effect, despite the very high TRIAC concentrations reached in heart. Cold exposure did not affect D1 activity in the former tissues. Similar results (higher $T_3$ effect) were found for D1 mRNA in liver and kidney (results not shown).

In BAT, D2 accounts for the local production of $T_3$. BAT D2 activity was suppressed by the lowest TRIAC dose (35% of control) and increased with the highest $T_3$ dose (>5-fold) and cold exposure (>8-fold). Pituitary D2 increased twofold with the highest $T_3$ dose. Pituitary D1 (86% of the total activity) did not change with any of the treatments (not shown). D2 in cerebral cortex was induced by both $T_3$ doses and by the highest TRIAC dose.

*Similar effect of TRIAC and $T_3$ in heart.* It has been shown that TRIAC has a lesser effect on the heart than $T_3$ (27, 53). For this reason, the expression of three genes (α- and β-MHC and HCN2) regulated in heart by $T_3$ was investigated (Fig. 9). Both, α- and β-MHC have been shown as regulated in opposite directions by hypothyroidism, with α-MHC levels decreasing and β-MHC levels increasing. These opposite changes are not always found after treatment with $T_3$ (46, 62). In our model, an inhibition of β-MHC was observed in a dose-dependent manner when using $T_3$ and TRIAC and by cold exposure. No difference was observed between $T_3$ and TRIAC. The effects on α-MHC are less intense, and an inhibition was only observed under cold exposure. The expression of HCN2 increased only after the high $T_3$ dose. LPL mRNA in heart was inhibited only by the highest $T_3$ dose and cold exposure (Fig. 6). In heart, $T_3$ concentrations were fourfold those of TRIAC. Unexpectedly, after infusion of 4 nmol TRIAC, the concentrations of TRIAC in heart were the highest of any of the tissues studied, similar to those of liver; despite this, the effects of TRIAC and $T_3$ were similar in heart.

## DISCUSSION

TRIAC is a physiological product of $T_3$ metabolism, with high affinity for the TR-$\beta_1$ isoform. Its biological functions are assumed to be similar to those of $T_3$, but it is not known whether TRIAC has specific but as yet unidentified roles. The present study provides evidence of specific effects of TRIAC different from those of $T_3$, especially in adipose tissue, in a model that does not use TRIAC as substitution therapy, because it is known that $T_4$ is required for the production of $T_3$. One of the best-characterized effects of TRIAC is the inhibition of TSH levels (3, 9, 16) accompanied in humans by low $T_4$ levels and persistent hypothyroidism. In our study, the infusion of both $T_3$ doses and the high TRIAC dose decreased $T_4$ concentrations in plasma and tissues and inhibited pituitary TSH and the thyroidal synthesis of $T_4$. However, the lowest dose of TRIAC had no effect on plasma TSH, although it

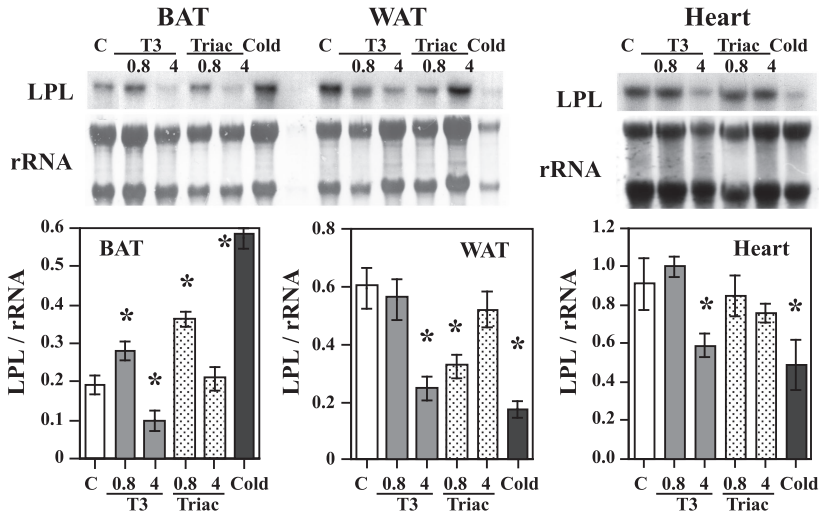


Fig. 6. Expression of lipoprotein lipase (LPL) in BAT, WAT, and heart. Total RNA was extracted from BAT, WAT, and heart of rats from groups described in Fig. 1. Hybridization with LPL, rRNA staining, and LPL/rRNA ratio are shown (means ± SE, $n$ = 2–3). *$P < 0.05$ vs. C.

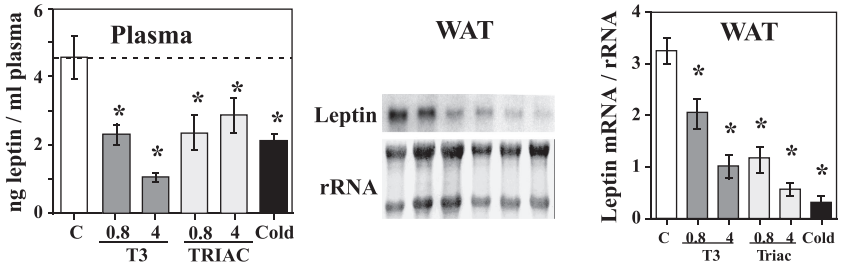


Fig. 7. Plasma leptin and leptin mRNA expression in WAT. Plasma leptin ($n$ = 4–5 rats/group), WAT leptin mRNA, rRNA staining, and leptin/rRNA ratio are shown ($n$ = 2; means ± SE). *$P < 0.05$ vs. C.

decreased plasma $T_4$ slightly, suggesting an effect of TRIAC on pituitary TSH, which did not affect $T_4$ concentrations in tissues. Therefore, TRIAC infusion seems less effective than $T_3$ infusion in inhibiting TSH, which is opposite to the findings in pituitary cells (16), probably due to a lower effect of TRIAC or lower TRIAC concentrations in the pituitary. Decreased plasma TSH follows the pattern of changes in pituitary TSH. This was already observed in hyperthyroid rats (42), in which the lack of response of TSH to thyrotropin-relasing hormone (TRH) was due to the complete depletion of pituitary TSH and not to high $T_3$ concentrations in the pituitary. The cold-exposed group is an exception to the parallel changes found in plasma and pituitary TSH. Whereas $T_3$ infusion caused a profound inhibition of TSH synthesis and secretion and possibly that of TRH, the adrenergic stimulation under cold exposure increased TRH secretion, leading to increases in TSH and plasma $T_3$ (and BAT $T_3$). Thus the stimulation of TRH overcame the inhibition by $T_3$. The low serum $T_4$ was followed by low $T_4$ in tissues in all the rats infused with $T_3$ and the highest TRIAC dose, but not in those on the lowest TRIAC dose. Although the decrease in $T_4$ in the tissues was less pronounced than in plasma, the tissue/plasma $T_4$ ratios were higher in the rats infused with both $T_3$ doses than in those infused with TRIAC. These data indicate that there is increased $T_4$ uptake to avoid tissue hypothyroxinemia.

The infusion of $T_3$ led to highly variable $T_3$ increases in the different tissues depending on the contribution of plasma-derived (liver, kidney) or locally produced $T_3$ (BAT).TRIAC concentrations have been reported in plasma (17, 35, 38), but our study presents the first data on TRIAC concentrations in rat tissues, which were always lower than those of $T_3$ (<10%). Except for decreases in heart, liver, and kidney when using the high TRIAC dose, TRIAC actions are achieved with no change in $T_3$ concentrations. Interestingly TRIAC is produced in WAT of $T_3$-treated rats and under cold exposure, an effect not observed in any other tissue, raising the possibility that TRIAC may have a role in regulating lipids and thermogenesis (leptin, UCP-1, or LPL expression) in WAT. The lower tissue-to-plasma ratios indicate that TRIAC does not enter as easily as $T_3$ into the tissues, especially under TRIAC infusion that decreases tissue-to-plasma ratios in most tissues, except in liver and kidney.

Our results disclose differences in the regulation of gene expression when using $T_3$ and TRIAC. The lower dose of TRIAC was more effective than $T_3$ in the upregulation of UCP-1 mRNA, whereas no effect was seen at the high dose. We show for the first time the induction of UCP-1 in BAT in vivo at very low TRIAC concentrations, as previously observed in cultured brown adipocytes (34). In the present model, UCP-1 induction occurs without exogenous adrenergic

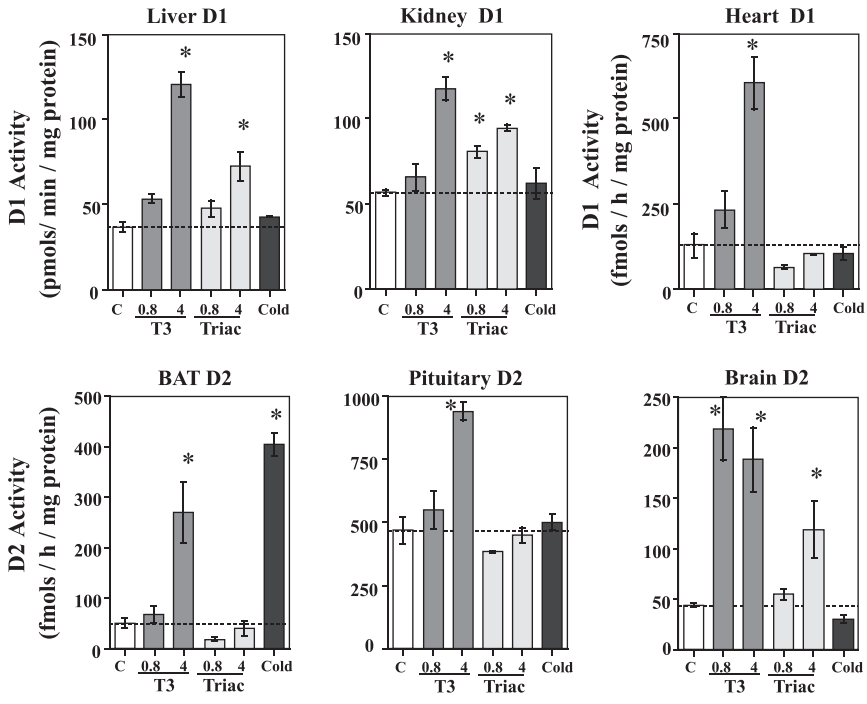


Fig. 8. Type I and II Deiodinase (D1 and D2) activities in liver, kidney, heart, BAT, pituitary, and brain. D1 activities in liver, kidney, and heart and D2 activities in BAT, pituitary, and brain (pmol·min$^{-1}$·mg protein$^{-1}$ for D1 in liver and kidney and fmol·h$^{-1}$·mg protein$^{-1}$ for D1 in heart and D2 in BAT, pituitary, and cerebral cortex in brain; $n$ = 5 / group; means ± SE). *$P < 0.05$ vs. C.

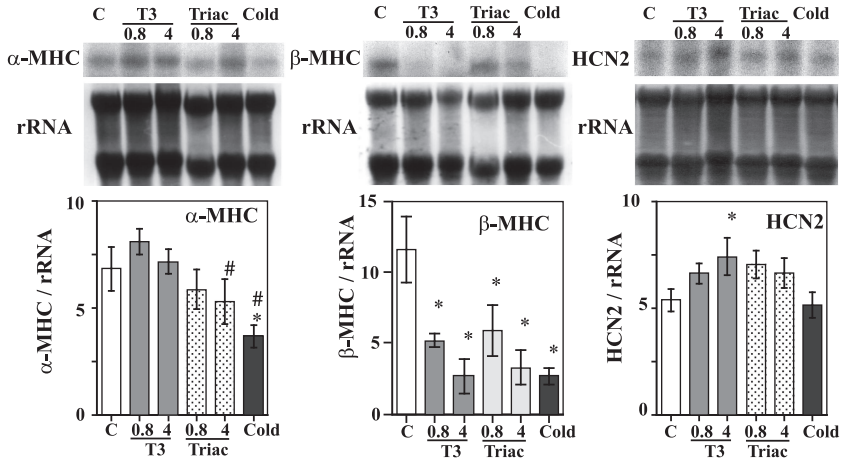


Fig. 9. Expression of α- and β-myosin heavy chain (MHC) and hyperpolarization-activated cyclic nucleotide-gated channel 2 (HCN2) mRNAs in heart. Total RNA was extracted from heart of rats from experimental groups described in Fig. 1. Hybridization with α- and β-MHC and HCN2, rRNA staining, and ratios to RNAs are shown (means ± SE, $n = 4$). *$P < 0.05$ vs. C and #$P < 0.05$ vs. 0.8 nmol $T_3$.

stimulation (injection of NE). The lowest TRIAC dose was as effective as the higher $T_3$ dose in the induction of UCP-2 and LPL in BAT, the inhibition of UCP-2 in muscle, and the inhibition of LPL and leptin mRNA in WAT. It is possible that TRIAC, acting through specific transcription factors, regulates several genes through common pathways. The effect of the low (but not high) doses of TRIAC was also observed in brown adipocytes in culture (34), increasing the adrenergic stimulation of UCP-1 and other genes such as D2, type III 5-deiodinase, and LPL. Another compelling link between TRIAC and thermogenesis was the induction of ectopic UCP-1 expression in WAT, an effect not observed with any other treatment. UCP-1 is considered a specific marker of BAT and thermogenic activity. Therefore, TRIAC was able to induce UCP-1 in the so-called "convertible" WAT, meaning WAT that is converted into BAT, by induction of UCP-1, as it happens under intense cold exposure (28), $\beta_3$-adrenergic stimulation, or leptin treatment (11, 19, 45). Other experimental models have also shown induction of UCP-1 in WAT, such as targeted disruption of the RII subunit of protein kinase A with decreased WAT mass (12) and the targeted disruption of the corepressor repressor interacting protein (RIP)140 (25). TRIAC at low concentrations may induce the release of corepressors inducing UCP-1, but this interaction remains to be demonstrated.

Several genes in liver and heart, with predominant TR-$\beta_1$ and -$\alpha_1$ isoforms, respectively, were also studied to explore whether the higher effect of TRIAC could be due to its higher binding to the TR-$\beta_1$ isoform. Because the induction of hepatic D1 was higher when using $T_3$, it seems that the effects of TRIAC at low doses do not depend on a higher abundance of the TR-$\beta_1$ isoform, because it is not found in liver. Experiments using hypothyroid mice treated with $T_3$ or the specific TR-$\beta_1$ ligand GC-1 showed that the stimulation of UCP-1 in BAT is mediated by the TR-$\beta_1$ isoform, but GC-1 failed to maintain core temperature under cold exposure, normalize heart rate, or increase the adrenergic response (49). Thus the higher effect of TRIAC observed here may be linked to a specific action on specific coactivators or corepressors in adipose tissue.

The study of the UCP family reveals that TRIAC regulates UCP-2 expression differently in each tissue. UCP-2 expression was increased in BAT and heart by $T_3$ and TRIAC but was decreased in muscle. The latter agrees with absent or minimal stimulation of UCP-2 by $T_3$ (24, 32), even after massive $T_3$ doses. The stimulation of muscle UCP-2 is only found when $T_3$ is given to thyroidectomized mice (21), but not in control mice. UCP-3 expression was only upregulated by the highest dose of $T_3$. Therefore, the UCPs appeared to be regulated independently.

To further study whether the actions of TRIAC at low doses were preferentially found in adipose tissue, two additional genes in adipose tissue (LPL and leptin) were examined. Effects of the lowest TRIAC dose on LPL were observed in BAT and WAT, although in opposite directions, but not in heart. For leptin, the inhibition was observed using $T_3$ and TRIAC, in agreement with previous results in cultured brown and white adipocytes (33), suggesting that LPL and leptin could contribute to increased energy expenditure or lipid mobilization in our model.

TRIAC (specially the low dose) had almost no effect in D1 activities, except in kidney. Indeed, the basal and $T_3$-induced expression of D1 is mostly dependent on TR-$\beta_1$, as shown using TR-$\beta_1$- and TR-$\alpha_1$-deficient mice (1), but whereas hepatic D1 is dependent on TR-$\beta_1$ by 70%, and in a small proportion on TR-$\alpha_1$, in kidney there is no role for the TR-$\alpha_1$ isoform. Kidney D1 is also less sensitive to $T_3$ regulation. The effect on D2 activity may be related to the decrease of $T_4$ in the tissues studied or to a stimulatory effect of $T_3$ itself (15, 31).

The observed effects of $T_3$ and TRIAC in heart do not give a clear explanation for the different response, possibly due to the action of $T_3$ on other end points. The inhibition of β-MHC was similar for $T_3$ and TRIAC, but little effect was found on the other genes studied, despite the high TRIAC concentration found in heart after TRIAC infusion (Fig. 3). Of note, $T_3$ concentrations in heart were reduced by 50% under the highest dose of TRIAC.

There were also many genes for which $T_3$ had more effect than TRIAC, such as the induction of D1 in liver, kidney, and heart, D2 in BAT, pituitary, and brain, UCP-3 in BAT and muscle, and LPL in heart. Conversely, some genes responded similarly to $T_3$ or TRIAC, for example UCP-2 and β-MHC in heart. The highest dose of $T_3$ produces a larger effect, whereas for TRIAC the larger effects are achieved using the smaller doses.

In conclusion, whereas the effect of TRIAC at the pituitary level has been well recognized, the effects on adipose tissue

had not been analyzed in detail yet, except for the actions on cholesterol and plasma lipids. Here we report the effect of low doses of TRIAC in inducing UCP-1 mRNA and possibly stimulating BAT and WAT thermogenesis. The ectopic expression of UCP-1 in WAT resembled other models in which there is activation of thermogenesis and energy expenditure. The effect of TRIAC at low doses on LPL or leptin reinforces its role in activating energy metabolism. In addition, all these effects are exerted without inhibition of TSH or hypothyroxinemia, contrary to the high doses of TRIAC. Thus, although the administration of high doses of TRIAC should be avoided, this study shows the physiological relevance of low doses of TRIAC inducing thermogenic effects in adipose tissues, suggesting that an increase in TRIAC production in adipose tissues may be one mechanism to increase energy metabolism and may be of benefit in the treatment of obesity.

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