Potential of brown adipose tissue type II thyroxine 5'-deiodinase as a local and systemic source of triiodothyronine in rats.

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Research Article

Previous reports suggest that a type II iodothyronine 5'-deiodinase may become the main enzymatic pathway for extrathyroidal triiodothyronine (T3) generation when the enzyme levels are sufficiently elevated and/or liver and kidney type I 5'-deiodinase activity is depressed. The present studies assessed the potential of brown adipose tissue (BAT) type II 5'-deiodinase to generate T3 for the plasma pool. BAT 5'-deiodination (BAT 5'D) was stimulated by either short- (4 h) or long-term (7 wk) cold exposure (4 degrees C). Long-term cold exposure increased thyroxine (T4) secretion 40-60% and extrathyroidal T3 production three-fold. In cold-adapted rats treated with propylthiouracil (PTU), extrathyroidal T3 production was 10-fold higher than in PTU-treated rats maintained at room temperature. Cold did not stimulate liver or kidney 5'D, but the cold-adapted rats showed a six- to eightfold higher BAT 5'D content. PTU caused greater than 95% inhibition of liver and kidney 5'D, but did not affect BAT 5'D. Thyroidectomized rats maintained on 0.8 micrograms of T4/100 g of body weight (BW) per day were acutely exposed to 4 degrees C. In rats given 10 mg of PTU/100 g of BW, 4 h of cold exposure still caused a 12-fold increase in BAT 5'D, a 2.3-fold increase in plasma T3 production, and a 4.8-fold increment in the locally produced T3 in BAT itself. All these responses were abolished by […]

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Abstract

Previous reports suggest that a type II iodothyronine 5'-deiodinase may become the main enzymatic pathway for extrathyroidal triiodothyronine (T_3) generation when the enzyme levels are sufficiently elevated and/or liver and kidney type I 5'-deiodinase activity is depressed. The present studies assessed the potential of brown adipose tissue (BAT) type II 5'-deiodinase to generate T_3 for the plasma pool. BAT 5'-deiodination (BAT 5'D) was stimulated by either short- (4 h) or long-term (7 wk) cold exposure (4°C). Long-term cold exposure increased thyroxine (T_4) secretion 40–60% and extrathyroidal T_3 production threefold. In cold-adapted rats treated with propylthiouracil (PTU), extrathyroidal T_3 production was 10-fold higher than in PTU-treated rats maintained at room temperature. Cold did not stimulate liver or kidney 5'D, but the cold-adapted rats showed a six-to eightfold higher BAT 5'D content. PTU caused >95% inhibition of liver and kidney 5'D, but did not affect BAT 5'D. Thyroidectomized rats maintained on 0.8 μg of T_4/100 g of body weight (BW) per day were acutely exposed to 4°C. In rats given 10 mg of PTU/100 g of BW, 4 h of cold exposure still caused a 12-fold increase in BAT 5'D, a 2.3-fold increase in plasma T_3 production, and a 4.8-fold increment in the locally produced T_3 in BAT itself. All these responses were abolished by pretreatment with the α_1-antiadrenergic drug prazosin. Regardless of the ambient temperature, liver 5'D activity was >90% inhibited by PTU. These results indicate that BAT can be a major source of plasma T_3 under suitable circumstances such as acute or chronic exposure to cold. Furthermore, BAT 5'D activity affects BAT T_3 content itself, suggesting that thyroid hormone may have a previously unrecognized role in augmenting the thermogenic response of this tissue to sympathetic stimulation. Such interactions may be especially important during the early neonatal period in humans, a time of marked thermogenic stress.

Introduction

It is recognized that a large fraction of the circulating triiodothyronine (T_3)[1] is generated extrathyroidally from thyroxine (T_4) 5'deiodination (1–3). T_4 5'-deiodination is an enzyme-catalyzed reaction that takes place in several tissues. Largely because of the abundance of the enzyme in liver and kidney, these organs have been considered the main anatomic source of the extrathyroidally generated T_3 (4). The enzyme present in liver and kidney has received considerable attention over the last decade. The reaction is uncompetitively inhibited by propylthiouracil (PTU), which is postulated to react with the sulfenyl iodide complex formed in the reaction (5, 6). The inhibition by PTU can be demonstrated either in vivo or in vitro (7, 8). Reduced enzyme activity can be demonstrated in fasting, hypothyroidism, and during the fetal and early neonatal period (9). We have termed this enzyme type I 5'-deiodinase (5'D-I).

PTU can decrease extrathyroidal T_4 to T_3 conversion by 60–70% in euthyroid rats (10, 11), but does not affect the acute inhibition of thyrotropin (TSH) release by T_4 (11, 12), or the pituitary and cerebral cortex content of [^{125}I]T_3 in animals injected with [^{125}I]T_4 (12, 13). Altogether, these findings led to the discovery of a second enzymatic pathway for T_4 5'-deiodination. The second enzyme, which we have termed type II 5'-deiodinase (5'D-II), when compared to 5'D-I, exhibits different kinetics, has an apparent K_m for T_4 that is at least 400-fold lower, is insensitive to PTU, increases markedly and rapidly after thyroidectomy, and is rapidly suppressed by the injection of thyroxine and its analogs (14–18).

The failure to abolish extrathyroidal T_4 to T_3 conversion with PTU, along with the finding of a second pathway for T_4 to T_3 conversion, led us to reexamine the source of the extrathyroidally produced plasma T_3. The studies showed that in adult and neonatal hypothyroid rats, all the T_3 produced after a physiologic or a tracer dose of T_4 was generated via the 5'D-II pathway (3, 19). In 2-wk-old euthyroid rats, >80% of the plasma T_3 generated from T_4, as opposed to 30–40% in adult rats (10, 11, 13), derived from 5'D-II activity (19). Thus far, 5'D-II had been found only in the central nervous system and the anterior pituitary, both of which seemed unlikely for generating circulating T_3 from T_4. The search for another anatomic source of T_3 that could explain those findings, showed that brown adipose tissue (BAT) contained an enzyme that was indistinguishable from the 5'D-II described in the central nervous system and adenohypophysis (20). BAT 5'D was also elevated in hypothyroid rats but, at variance with the pituitary and cerebral deiodinase, BAT 5'D could be stimulated by the sympathetic nervous system as well (21). More recently, Goswami and Rosenberg (22) have reported that, at low substrate concentration, kidney microsomes can catalyze 5'deiodination of T_4 or reverse T_3 (rT_3) in the presence of 100 μM PTU, which opens the possibility of another "PTU-insensitive" extrathyroidal source of T_3. However, neither the physiologic significance of their findings nor the physiologic responses of this enzyme have been established.

The goal of the experiments to be described was to assess the potential of BAT to produce T_3 for the plasma pool. We

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1. *Abbreviations used in this paper:* AOV, one-way analysis of variance; BAT, brown adipose tissue; 5'D-I, type I 5'-deiodinase; 5'D-II, type II 5'-deiodinase; PTU, propylthiuracil; rT_3, reverse T_3, 3',5',3-triiodothyronine; T_3, triiodothyronine; T_4, thyroxine; TWAOV, two-way analysis of variance.

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have taken advantage of the possibility of manipulating the enzyme activity levels through the sympathetic nervous system and the relative insensitivity of the enzyme to inhibition by PTU. The results of the present study reveal that BAT 5'D not only can produce significant amounts of T_3 for the plasma pool, but also can augment markedly the T_3 content of BAT itself.

Methods

These studies were performed on Sprague-Dawley rats obtained from Zivic-Miller Laboratories, (Allison Park, PA). At the beginning of the experiments the weight of the animal ranged from 75 to 100 g or 130 to 150 g as indicated. In some experiments, rats were thyroidectomized and placed on 0.5% calcium chloride in the drinking water. T_4 was replaced as described along with the experiments. The rats were maintained at 23±1°C or at 4±1°C as indicated in the text. They were given tap water and rat laboratory diet ad libitum, and were submitted to light cycles of 14 h regardless of the ambient temperature.

Tracers. $[^{125}I]T_4$ (sp act ~ 4,200 μCi/μg) and $[^{131}I]T_3$ (sp act ~ 2,800 μCi/μg) were prepared from T_3 and 3,5-L-diiodothyronine, respectively, as previously described (23, 24). The radiolabeled iodothyronines were prepared within 48 h of the experiment and contained <5% iodide as a major contaminant; $[^{125}I]T_4$ had ≤0.2% $[^{125}I]T_3$ and $[^{131}I]T_3$ < 0.5% $[^{131}I]T_4$, as determined by a combination of affinity and paper chromatography (25, 26). Tracers were stored in 70% ethanol. Immediately before the injections they were rapidly dried under a stream of nitrogen and redissolved in 10% rat serum in 0.9% sodium chloride containing 0.1% of sodium iodide (3, 19). The tracers, 30–80 μCi of $[^{125}I]T_4$ and 9–20 μCi of $[^{131}I]T_3$ as indicated, were injected into one of the external jugular veins in 0.1–0.2 ml of that vehicle.

Collection of sera and tissue samples. Rats were lightly anesthetized with ether, and 0.3–0.5 ml of blood was drawn from the heart at the indicated times after injecting its tracers. A blood sample was always obtained immediately before these injections. Rats were killed by exsanguination through the aorta under ether anesthesia. Interscapular BAT and aliquots of liver and kidney were collected for the measurement of either the content of labeled iodothyronines or 5'-deiodinases. The tissues were immediately frozen in dry ice/acetone and analyzed between 24 and 48 h of the experiment. The blood was allowed to clot, and the serum was collected and frozen until the measurements of labeled or unlabeled iodothyronines were performed.

Measurements of tracers in the serum. Serum $[^{125}I]T_4$ and $[^{131}I]T_3$ were separated and quantitated by paper chromatography (3, 19). The origin of the Whatman 3 MM (Whatman, Inc., Clifton, NJ) was moistened with 25 μl of ethanol, and while wet, 20 μl of serum was applied followed by 50 μl of "marker" (~2 mg/ml T_4, T_3, and sodium iodide in ethanol made alkaline by adding 5% concentrated NH_4OH). This volume of serum directly applied to the chromatography paper had no effect on the resolution of the major peaks of radioactivity (3, 19). $[^{125}I]T_3$ derived from $[^{125}I]T_4$, $[^{125}I]T_3(T_4)$, was measured by a combination of affinity and paper chromatography as described previously (3, 25, 26). The concentration of $[^{131}I]T_3$ was determined from the total ^{131}I counts recovered as $[^{131}I]T_3$ in paper chromatography. The $[^{131}I]T_3$ concentration, expressed as a fraction or percentage of the injected $[^{131}I]T_3$, was used for calculating T_3 kinetics parameter (see below), as an internal recovery standard for the $[^{125}I]T_3$, and to estimate the fraction of the observed $[^{125}I]T_3$ that was derived from the minute amounts of $[^{125}I]T_3$ contaminating the $[^{125}I]T_4$ dose (3, 12). This latter correction factor was negligible by 4 h after the injection (3, 12). The endogenously generated $[^{125}I]T_3(T_4)$ was then multiplied by 2 to correct for the halving of the T_4 specific activity during conversion to T_3. For the calculation of the mass of T_3 derived from T_4, an additional correction derived from the change in molecular weight was made, the factor 651/777. The concentration of all three radioactive iodothyronines was expressed as a percentage of the corresponding injected amounts.

Measurement of labeled iodothyronines in the BAT. The tissue was weighed and rapidly counted before complete thawing. Subsequently, it was manipulated and extracted as described previously (27, 28) with minor variations, namely the use of butanol: 2 M acetic acid instead of butanol/HCl. With this modification we extracted >90% of the BAT counts in two extractions with 2 and 1 ml of butanol, respectively. There was no significant degradation of radiolabeled T_3 and there was <10% degradation of $[^{125}I]T_4$, which was recovered as iodide and as unidentified radioactivity in the solvent front of the chromatogram (28). No correction was done for the plasma trapped in the BAT, because the volume trapped accounted for <10% of the $[^{125}I]T_3(T_4)$ counts observed in this tissue (29). The tissue content of $[^{125}I]T_3$ was also corrected by the $[^{125}I]T_3$ contaminating the $[^{125}I]T_4$ dose, although 4 h after the injection this figure was negligible (<2% of the observed $[^{125}I]T_3$ concentration). The endogenous $[^{125}I]T_3(T_4)$ was then corrected for the specific activity and molecular weight changes, as appropriate.

Kinetic analysis. The experimental protocols are described with results. T_4 and T_3 clearances, fractional rates of disappearance from plasma, and fractional T_4 to T_3 conversion were calculated by noncompartmental analysis as described previously (3, 19). Details applicable to the experiments are described along with the results. For convenience, in the studies to be described, the number of samples and the length of the interval of sampling were reduced to a minimum. This may result in some limitations in extrapolating the data to steady-state conditions, but it does not affect the conclusions of the present experiments.

Other procedures. Serum T_4 and T_3 concentrations were measured by previously described methods (30). Iodothyronine-stripped rat serum instead of human serum was used for the standard curves in the radioimmunoassay.

Data were statistically analyzed by Student's t test and by one- or two-way analysis of variance, AOV and TWAOV, respectively. For multiple comparisons, when AOV indicated significant differences, the Newman–Keuls test was used (31).

Results

Effects of chronic cold exposure on thyroid hormone production and iodothyronine deiodinases. Euthyroid rats weighing 140±8 g (SEM) were divided into two groups. One was kept at ~23°C and the other at 4±1°C for 7 wk. Both groups had free access to water and food, but the cold-exposed group gained less weight: at the end of the 7-wk period they weighed 425±18 g vs. 540±13 g ($P < 0.001$). At the end of 7 wks, one-half of the rats in each group were injected intraperitoneally with PTU 3 mg/100 g BW, followed 30 min later by ~30 μCi of $[^{125}I]T_4$ and ~9 μCi of $[^{131}I]T_3$ in a jugular vein. PTU injections were repeated ~17 h later. This dosage of PTU ensured full inhibition of liver and kidney 5'-deiodinases (3, 13). Rats were bled ~0.5 ml from the heart under light ether anesthesia 17, 20, and 23 h after the tracer injection. From the curves subtended by these time points, we obtained the areas under the curves within this interval, the fractional disappearance rate of T_4 and T_3, the amount of tracers remaining at the time of starting the bleedings, and the fraction of the tracers consumed during the interval from 17 to 23 h. Total production rates of T_4 and T_3 were calculated from these parameters and the serum concentration obtained at the time of injecting the tracers based on principles described previously (3, 19, 32, 33):

$$Cl = \frac{100(e^{-\lambda t_1} - e^{-\lambda t_2})}{\int_{t_1}^{t_2} C dt}, \quad (1)$$

where Cl = plasma clearance rate; λ = fractional removal rate of $[^{125}I]T_4$ or $[^{131}I]T_3$, as determined from the individual curves; t_1 and t_2 = the beginning and the end of the interval of observation; and C = serum concentration of $[^{125}I]T_4$ or $[^{131}I]T_3$.

$$PR = Cl \times \text{Serum } T_3 \text{ or } T_4 \text{ (RIA) concentration}, \quad (2)$$

where PR = production rate.

Fractional T_4 to T_3 conversion, FC, was calculated from the integrated $[^{131}I]T_3$ and $[^{125}I]T_3(T_4)$ concentrations as discussed previously (3, 19) based on principles analyzed in detail elsewhere (32):

$$FC = \frac{100(e^{-\lambda_3 t_1} - e^{-\lambda_3 t_2})}{\int_{t_1}^{t_2} [^{131}I\text{-}T_3] \cdot dt} \times \int_{t_1}^{t_2} [^{125}I\text{-}T_3(T_4)] \cdot dt, \quad (3)$$

where λ_3 = fractional removal rate of $[^{131}I]T_3$ as determined individually; t_1 and t_2 are the beginning and the end of the interval of observation; and $[^{131}I\text{-}T_3]$ and $[^{125}I\text{-}T_3(T_4)]$ are the serum concentrations of $[^{131}I]T_3$ and $[^{125}I]T_3(T_4)$, which in Eq. 3 have been integrated between t_1 and t_2.

The integrated concentration of $[^{131}I]T_3$ was calculated from the slope and the serum concentrations as determined individually, and for $[^{125}I]T_3$, it was obtained by direct measurements of the area subtended by the individual curves.

$$PR_{T_3(T_4)} = FC \times PR_{T_4} \times \frac{651}{777}, \quad (4)$$

where $PR_{T_3(T_4)}$ and PR_{T_4} are the production rates of T_3 from T_4 and of T_4, respectively; FC = fractional T_4 to T_3 conversion; and 651/777 a factor to correct for the change in molecular weight derived from the removal of one atom of iodine from T_4.

The mean±standard error of the mean serum concentrations of $[^{125}I]T_4$, $[^{125}I]T_3(T_4)$ and $[^{131}I]T_3$ are shown in Fig. 1. The effects resulting from cold exposure on serum hormone concentrations and of cold and PTU on T_4 and T_3 productions are summarized in Table I. The data were analyzed by AOV and TWAOV. Cold exposure did not affect serum T_4 concentrations, but serum T_3 levels were elevated in cold-exposed animals. Cold exposure induced a modest but significant increment in T_4 clearance, whereas that of T_3 was not affected. PTU did not significantly affect the clearance of either hormone. T_4 production rate and total T_3 production were higher in cold-exposed rats ($P < 0.001$ and < 0.0025). Cold-acclimation also increased the fractional T_4 to T_3 conversion by threefold. Whereas in the rats maintained at 25°C we observed the usual marked inhibition of T_4 to T_3 conversion by PTU (~75%), this was of less magnitude in the cold-exposed animals (~50%). The higher rates of fractional conversion of T_4 to T_3 and production of T_4 in cold-exposed animals resulted in an approximate fourfold increment in extrathyroidal T_3 production. Most notably, in the cold-exposed animals there was a 10-fold greater PTU-insensitive T_3 generation from T_4 than in the animals kept at normal ambient temperature.

It is important to stress that in these experiments the figures for production rates, fractional T_4 to T_3 conversion, and T_3 secretion vs. extrathyroidal T_3 production in the control animals are in good agreement with those previously reported (2, 3, 8), in spite of the differences in age of the animals and the abbreviated period of sampling in the present experiments. In the cold-adapted rats, all the T_3 production appears as extrathyroidal. This probably results from the accumulated dispersion of the data along the calculations which makes the thyroidal T_3 secretion inapparent.

The effect of the cold and PTU on liver and kidney deiodinases is shown in Table II. These assays were performed in the same animals 2 wk after the studies described above. The ambient conditions were rigorously maintained and PTU—but not the tracers—were administered in the same dosage schedule described. Low ambient temperature did not affect the enzyme levels in either tissue, whereas PTU inhibited the enzyme >98% in cold-exposed animals, as it does in rats at normal ambient temperature (3, 13).

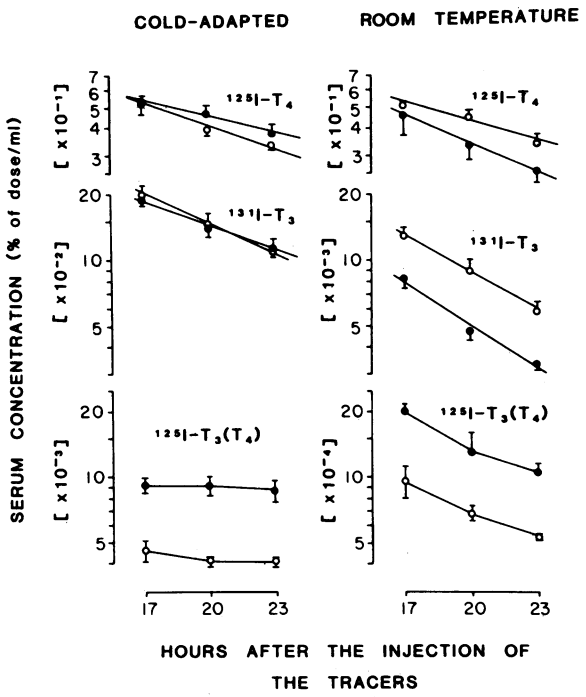

Figure 1. Serum concentrations of $[^{125}I]T_4$, $[^{131}I]T_3$, and $[^{125}I]T_3(T_4)$ 17, 20, and 23 h after injecting $[^{125}I]T_4$ and $[^{131}I]T_3$ into cold-adapted or age-matched control rats maintained at room temperature. (●) Control animals; (○) animals pretreated with PTU. Results have not been normalized by body weight. Cold-adapted control: 437±22 g (SEM); cold-adapted + PTU: 414±31 g; room temperature: 629±34 g; and room temperature + PTU: 640±23 g. Note the differences in the various scales. Experimental protocol described in detail in the text.

BAT 5′deiodinase activities measured in the same rats are shown in Fig. 2. The specific activity of the enzyme was about twofold increased in the cold-adapted animals and it was not affected by the PTU administration. Because of the twofold increment in BAT mass (763±74 vs. 354±64 mg, $P < 0.001$), the 2.3-fold increment in protein content (58±6 vs. 25±2 mg/g of BAT in the 1,500-*g* supernatant, $P < 0.001$), and the lower body weight of the cold-adapted animals, the BAT 5′D per 100 g BW resulted greater than sixfold higher than in the room temperature controls. These changes in BAT mass and protein content have been reported previously (34).

Effects of acute cold exposure on T_3 production in thyroidectomized T_4-replaced PTU-treated rats. Effects of α_1-adrenergic blockade. The studies described above suggest that T_3 production increases in cold adaptation, largely as a consequence of more active extrathyroidal T_3 production and, further, that the enzymatic source is not the liver or kidney 5′D-I but more likely a tissue such as BAT containing a PTU-insensitive, cold-stimulated 5′deiodinase. The following experiments were performed to delineate more precisely the source of T_3, i.e., to test the hypothesis as to whether the BAT 5′deiodinase was the source

Table I. Effects of Chronic Low Ambient Temperature on Thyroid Hormone Production and on the Effect of PTU on Extrathyroidal T_3 Generation

Condition	PTU	Serum hormone concentration		Plasma clearance		Total production rate		Fractional T_4 to T_3 conversion	Extrathyroidal T_3 production
		T_4	T_3	T_4	T_3	T_4	T_3		
		ng/ml		*ml/h · 200 g BW*		*ng/d · 200 g BW*		*%*	*ng/d · 200 g BW*
Ambient temperature									
23°C	−	40±4	0.44±0.05	1.3±0.2	50±13	1,230±166	531±146	16±2	191±30
23°C	+	36±3	0.40±0.03	1.2±0.1	32±4	1,064±111	306±16	4±0.3	39±5
4°C	−	41±4	0.68±0.06	1.8±0.2	45±3	1,752±184	736±99	52±8	774±50
4°C	+	37±4	0.58±0.09	2.0±0.1	47±5	1,725±135	653±118	25±8	377±83
Statistical analyses									
AOV		NS	NS	<0.025	NS	<0.025	NS	<0.001	<0.001
TWAOW									
Temp		NS	<0.005	<0.001	NS	<0.001	<0.025	<0.001	<0.001
PTU		NS	NS	NS	NS	NS	NS	<0.001	<0.001

Each entry is the mean±standard error of the mean of four rats. Euthyroid rats weighing initially 140 g (120–160 g range) were used. One-half were housed at ~23°C and the other at 4±1°C for 7 wk. The day before the experiment, a blood sample was obtained and they were subsequently injected intraperitoneally with PTU 3mg/100 g BW. Tracers ([^{125}I]T_4 and [^{131}I]T_3) were injected intravenously 30 min later and the injection of the PTU repeated 17 h later. At the time of the experiments, cold-exposed rats weighed 425±18 g (SE) and the controls 540±12 ($P < 0.001$).

of the excess extrathyroidal T_3 seen in response to the stimulus of cold. Our basic strategy has been to use specific stimulation, cold, and inhibition by the α_1-antiadrenergic agent prazosin, to manipulate BAT 5'deiodinase and, in addition, to examine the T_3 content in BAT in response to these manipulations and the 5'D-I activity in liver and kidney. To avoid the problem of T_3 secretion in the analyses, rats were thyroidectomized. The experimental protocol is shown in Fig. 3. 20 rats were thyroidectomized and started the following morning on T_4 replacement, 0.8 μg/100 g of BW per day subcutaneously (11). All animals were kept at room temperature and allowed to recover for a week. In the morning of the eighth day, 10 rats were kept at room temperature and the other 10 put in the cold room at 4±1°C for 4 h. One-half of the animals kept at room temperature and all the animals going to the cold room received 5 mg/100 g of BW PTU intraperitoneally the night before and at the time of initiating the cold stress. 5 of the 10 rats submitted to cold stress received, in addition, 0.4 mg/100 g of BW prazosin intraperitoneally at the start of the cold exposure, and boosters of 0.2 mg/100 g of BW 2, 4, and 6 h later. This dosage fully prevented the cold-induced elevation of BAT 5'deiodinase in previous experiments (21), but did not affect liver or kidney deiodinase (liver 1.46±0.2 vs. 1.40±0.2, kidney 1.70±0.1 vs. 1.99±0.1, all as nmol rT_3/h · mg protein as tested in preliminary experiments). At the time of initiating the cold stress, a blood sample was obtained from a jugular vein and subsequently all the rats were injected with ~80 μCi of [^{125}I]T_4 + 1.08 μg T_4 and ~20 μCi of [^{131}I]T_3. This dose of T_4 represents a mean of 0.84 μg/100 g of BW per rat. Blood samples (~0.3 ml) were obtained

Table II. Liver and Kidney 5'D-I Activities in Rats Exposed to Cold for 7 wk: Effect of Pretreatment with PTU

Condition	Liver	Kidney
	5'D-I (nmol I/h · mg protein)	*5'D-I (nmol I/h · mg protein)*
23°C	3.30±0.42	3.80±0.16
4°C	3.16±0.50	3.17±0.11
4°C + PTU	0.05±0.01	0.04±0.11

Rats from experiment shown in Table I were maintained under the same conditions for 2 wk, when they were handled and treated exactly as before but omitting the injections of tracers. They were killed at the same time after the first PTU injection and liver and kidney 5'-deiodinase activities were measured as described in Methods.

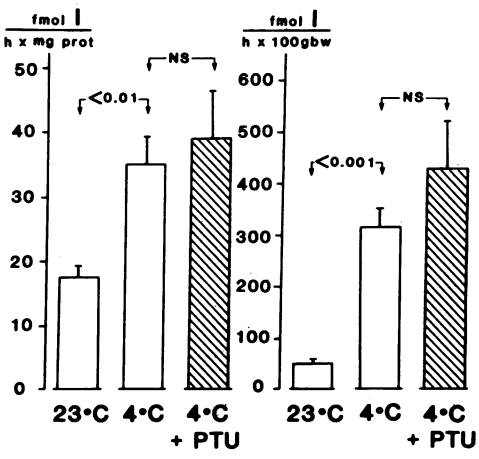

Figure 2. Interscapular BAT 5'deiodinase activity in rats exposed to 4°C for 7 wk. Enzyme was assayed as described in Methods using [^{125}I]rT_3 as substrate in the presence of 1 mM PTU. In the right panel the activity has been expressed on a body weight basis (gbw), because cold exposure resulted in a marked increment in the mass and protein content of BAT.

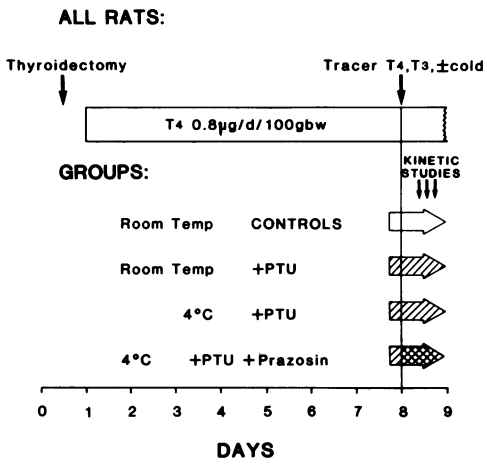

Figure 3. Experimental protocol to explore BAT T_3 production in rats acutely exposed to cold. The dosage and timing of PTU and prazosin are given in Results.

from the heart 4, 6, and 8 h after the injections. The concentrations of $[^{125}I]T_4$, $[^{125}I]T_3(T_4)$ and $[^{131}I]T_3$ were plotted (Fig. 4) and the clearances of $[^{131}I]T_3$ and $[^{125}I]T_4$ calculated from the fraction of tracers metabolized during the interval of observation and from the areas subtended by the individual curves (3, 19, 32). Because the tracers were injected along with the daily replacement dose of T_4, the T_3 newly formed was calculated from the specific activity of the dose. The resulting figure does not represent the total production of T_3 during this interval in that the amount of T_4 remaining from previous doses was not taken into account. Rat weight at the time of injecting the tracers was 129±2 g (SEM) and the weight gain during the week of treatment was 32±1 g, with no significant differences among the four groups. Similarly, serum T_4 and T_3 levels at this time did not differ among groups and were 1.7±0.09 (SEM) µg/dl and 0.30±0.06 ng/ml, respectively. These are values obtained ~24 h after the previous replacement dose. Sham thyroidectomized rats gained 39±11 g (n = 6), not significantly different from these rats, whereas untreated thyroidectomized rats, with undetectable serum T_4, gained 15±3 g ($P < 0.01$).

Serum levels of $[^{125}I]T_4$, $[^{125}I]T_3(T_4)$ and $[^{131}I]T_3$ at the various times sampled after the injection of tracers are presented in Fig. 4. The concentrations are expressed as percentage of the dose per milliliter of serum and the means±standard error of the mean plotted in a log scale. The data derived from the individual curves, and the calculated fractional T_4 to T_3 conversion and T_3 production from the dose are presented in Table III. They correspond to the actual interval between 4.3 and 8.3 h after the injection of the tracers. The data have been analyzed by AOV and by the Newman–Keuls test. None of the experimental conditions affected the fractional disappearance of the tracers nor the fraction of $[^{131}I]T_3$ or $[^{125}I]T_4$ consumed during the interval of observation. The areas under the curves of $[^{131}I]T_3$ were affected by all the experimental conditions, reflecting changes in the plasma clearance of T_3, which can be obtained by dividing the percentage of T_3 consumed during the interval by the area under the serum concentrations (3, 19, 32, 33 and Eq. 1). Thus, PTU resulted in significant reductions of $[^{131}I]T_3$ clearances as reflected by the areas under the curves. It is essential to take into account these variations in T_3 clearance to calculate T_3 production from T_4, as summarized by Eq. 3.

The most relevant results of this experiment are shown in the last two columns of Table III. PTU decreased fractional conversion by 70% at room temperature, as has been shown repeatedly (10, 11, 13). In marked contrast, the PTU-treated rats exposed to cold did not show a reduction in fractional conversion when compared to controls at room temperature. In the

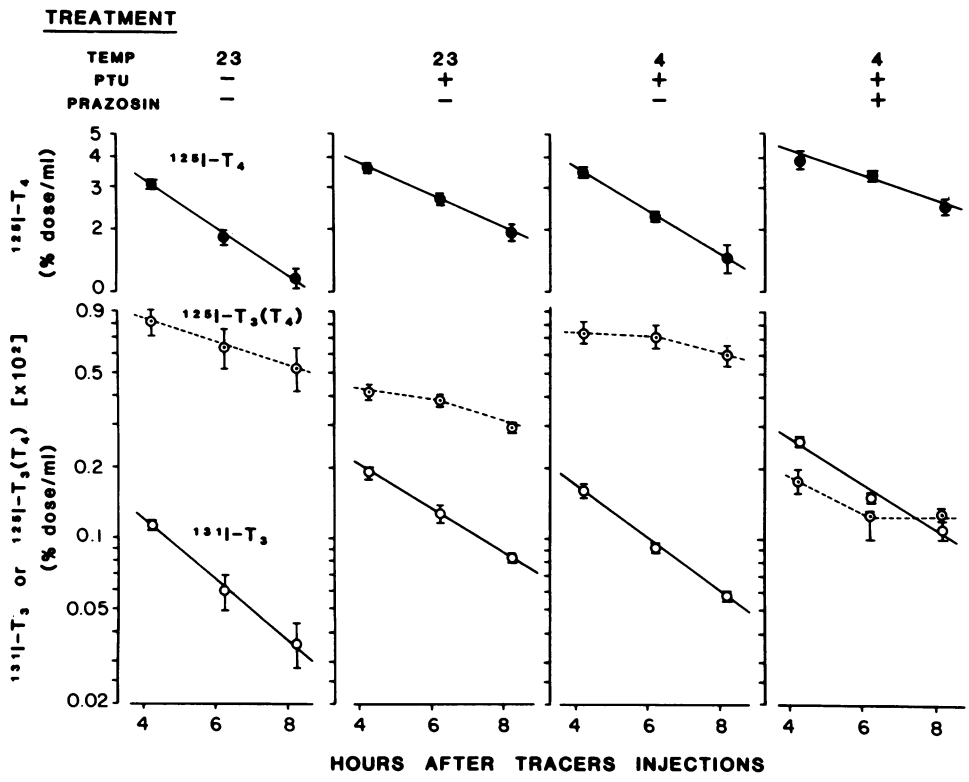

Figure 4. Serum concentrations of $[^{125}I]T_4$, $[^{131}I]T_3$, and $[^{125}I]T_3(T_4)$ at various times after injecting $[^{125}I]T_4$, 1,080 ng/rat, and tracer $[^{131}I]T_3$ into rats acutely exposed to cold. The experimental protocol is that schematically shown in Fig. 3. These are the data bases used to do the calculations, the results of which are shown in Tables III and IV. The concentrations have not been normalized to body weight. Mean±SE body weights at the time of injecting the tracers were: control, 140±4; room temperature + PTU, 138±5; cold + PTU, 144±6; and cold + PTU + prazosin, 145±3, not different by AOV.

Table III. Effects of Ambient Temperature, PTU, and Prazosin on T_4 to T_3 Conversion in Thyroidectomized Rats Replaced with T_4

	Condition	λ_3	Fraction of $[^{131}I]T_3$ consumed	Area under $[^{131}I]T_3$	λ_4	Fraction of $[^{125}I]T_4$ consumed	Area under $[^{125}I]T_3(T_4)$	Fractional conversion	$T_3(T_4)$ produced
		h^{-1}		$(\%/ml)\cdot h$	h^{-1}		$(\%/ml)\cdot h \times 10^{-4}$	%	ng/200 g
A.	Control	−0.30±0.04	0.19±0.02	0.26±0.04	−0.22±0.01	0.22±0.01	263±48	8.7±1.2	26.0±4.5
B.	23°C + PTU	−0.21±0.02	0.23±0.00	0.52±0.03	−0.17±0.01	0.24±0.00	150±9	2.7±0.1	8.5±0.2
C.	4°C + PTU	−0.26±0.03	0.21±0.01	0.39±0.02	−0.21±0.03	0.22±0.01	283±20	6.8±0.4	19.0±0.5
D.	4°C + PTU + PRA	−0.20±0.01	0.23±0.00	0.66±0.02	−0.15±0.02	0.23±0.01	59±12	0.9±0.1	2.6±0.5
	AOV								
	F ratio	2.91	2.86	25.05	1.94	1.08	9.63	19.63	13.34
	P	NS	NS	<0.001	NS	NS	<0.003	<0.001	<0.001
	Significant by NK test	—	—	ALL	—		*A* vs. *B* *C* vs. *D* *B* vs. *C*	*A* vs. *B* *C* vs. *D* *B* vs. *C*	*A* vs. *B* *C* vs. *D* *B* vs. *C*

Each entry is the mean±SEM of the values calculated individually for each rat for the interval between 4.3 and 8.3 h after the injection of the tracers. The mean curves of a serum concentration as function of time are shown in Fig. 3. The mean dose of T_4 injected with the tracer was 0.84 μg/100 g BW (1,080 μg/rat). Fractional conversion and production rate of T_3 for the interval can be calculated with the other data as explained in the text. The SEM values 0.00 mean that the next significant figure was <5, but they are not shown for convenience. Abbreviations: λ, fractional removal rate for $[^{131}I]T_3$ (λ_3) and $[^{125}I]T_4$ (λ_4); PTU, propylthiouracil; PRA, prazosin; NK, Newman-Keuls test.

cold-exposed PTU-treated animals, however, prazosin decreased fractional conversion by 87%. Because the fraction of the dose consumed during the interval of observation was not different, and the dose of T_4 was the same from all the animals, the amounts of T_3 produced and appearing in the plasma pool reflected basically the fractional conversions. In the cold-stressed PTU pretreated rats, the quantity of T_3 produced was not different from control animals, and was greater than twofold higher than in PTU-treated rats maintained at room temperature (compare rows *B* and *C* in Table III). Notably, 86% of this PTU-insensitive T_3 production was eliminated by prazosin pretreatment.

The 5'deiodinase activities in BAT and liver in these animals are shown in Fig. 5. BAT 5'deiodinase was not affected by PTU given in vivo, but was >10-fold increased by acute cold exposure (F ratio = 77.99, $P < 0.001$ by the Newman–Keuls test). This increment was abolished by treatment with prazosin, as previously reported (21). In contrast, regardless of the ambient temperature, hepatic 5'deiodinase was >90% inhibited by pretreatment with PTU. Prazosin had no additional inhibitory effect on this enzyme in the cold-exposed animals.

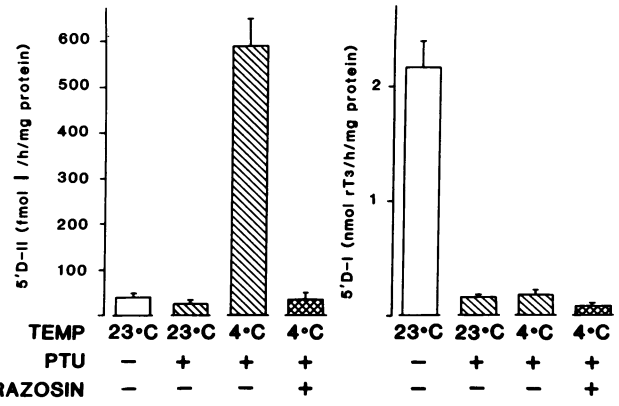

Figure 5. Effects of PTU, cold and prazosin on BAT and liver 5'deiodinase activity. Enzyme activity was measured as described in Methods. Newman–Keuls test showed that cold exposed-PTU-treated rats had significant higher BAT 5'D activity and that there were no differences among the other groups. By the same test, liver activity was significantly decreased by PTU regardless of the other experimental conditions; the differences among the groups receiving PTU were not significantly different.

The concentrations of the various radioactive iodothyronines and the calculated mass of T_3 in BAT derived from the dose of T_4 are presented in Table IV. Cold-stress resulted in a significantly higher $[^{125}I]T_4$ concentration in the BAT. This seemed the combined consequence of a higher BAT/serum ratio of $[^{125}I]T_4$ (not statistically significant), higher plasma $[^{125}I]T_4$ in the cold-stressed prazosin-treated rats and, presumably, a larger volume of plasma trapped in the BAT of cold-stressed animals. Therefore, is hard to attribute significance to this effect of acute cold exposure and remains open whether, if real, it might have contributed in part to the higher $[^{125}I]T_3(T_4)$ observed in the BAT of the cold-exposed rats. The concentration of $[^{125}I]T_3(T_4)$ in BAT was threefold higher in the cold-exposed PTU-treated rats than in the controls and eightfold greater than in the room temperature PTU-treated rats. The latter difference was abolished by pretreatment with prazosin. In contrast, the $[^{131}I]T_3$, i.e., the T_3 arriving in the tissue via plasma, was not higher in the cold-exposed PTU-treated rats. In the rats given prazosin, the higher $[^{131}I]T_3$ BAT content was a reflection of the higher serum levels, as shown in Fig. 4. Note that the BAT/serum ratio was not different in this group (Table IV).

The comparison of the BAT/serum ratios for $[^{125}I]T_3(T_4)$ and for $[^{131}I]T_3$ reveals highly significant differences. The higher is the former over the latter, the higher the fraction of locally produced T_3 over plasma hormone T_3. For convenience, the ratio of these two ratios has been entered as R in Table 4. First, R was >1 in all four groups ($P < 0.001$ by paired t test). Secondly, R was the highest in cold-exposed PTU-treated animals which was entirely accounted for in a higher $[^{125}I]T_3(T_4)$ BAT/serum ratio than in other groups ($P < 0.001$). Thirdly, prazosin abolished these differences between cold-exposed rats and those

Table IV. Effects of Ambient Temperature, PTU, and Prazosin on BAT T_4 and T_3 Concentrations after a Replacement Dose of $[^{125}I]T_4$

		Isotope concentration			BAT/serum ratio				T_3 content	
	Condition	$[^{125}I]T_4$	$[^{125}I]T_3(T_4)$	$[^{131}I]T_3$	$[^{125}I]T_4$	$[^{125}I]T_3(T_4)$	$[^{131}I]T_3$	R	Total	Local
		%/g	*%/g*	*%/g*	*ml/g*	*ml/g*	*ml/g*		*ng/g*	*ng/g*
A.	Control	0.41±0.02	0.11±0.01	0.29±0.04	0.33±0.02	25.6±7.3	8.9±1.2	2.9±0.7	0.98±0.08	0.57±0.11
B.	23°C + PTU	0.48±0.03	0.04±0.00	0.43±0.04	0.25±0.03	14.7±2.1	5.4±0.5	2.8±0.2	0.38±0.04	0.24±0.06
C.	4°C + PTU	0.82±0.15	0.33±0.03	0.34±0.07	0.41±0.14	57.1±8.3	4.9±0.2	11.5±1.3	3.03±0.29	2.76±0.30
D.	4°C + PTU + PRA	1.31±0.07	0.03±0.00	0.94±0.13	0.48±0.03	24.1±5.7	5.8±0.5	4.0±0.7	0.29±0.05	0.22±0.05
	F ratio	32.75	84.28	15.03	2.51	8.21	5.46	26.45	84.43	68.67
	P	<0.001	<0.001	<0.001	NS	<0.005	<0.02	<0.001	<0.001	<0.001
	Significant by NK test	All but *A* vs. *B*	All but *B* vs. *D*	*D* from all	—	*C* from all	*A* from all	*C* from all	*C* from all *D* vs. *A*	*C* from all

Each entry is the mean±SEM for the same rats the serum data of which are presented in Table III. Rats were killed 8.3 h after the tracer injection. The extraction and quantitation of the various iodothyronines are explained in Methods and the experimental protocol in Results. Abbreviations as for Table III. Total T_3 was calculated from the specific activity of the dose (100% = 1080 ng T_4). Local T_3 is the tissue $T_3(T_4)$ in excess of that obtained from the individual plasma $T_3(T_4)$ times the BAT/serum ratio of $[^{131}I]T_3$. *R* is the ratio of the $[^{125}I]T_3(T_4)$ BAT/serum ratio over that of $[^{131}I]T_3$.

maintained at room temperature. Altogether, these data indicate that there is a significant fraction of [^{125}I]T_3 in BAT that is unaccounted for by plasma T_3, i.e., that is the result of local production. Furthermore, the locally produced T_3 is markedly higher in animals acutely exposed to cold, a difference that is due to a PTU-insensitive, but prazosin-sensitive process, as is the stimulated BAT 5'D enzyme activity (Fig. 5).

With the [^{131}I]T_3 BAT/serum ratios and the plasma and tissue concentrations of [^{125}I]T_3(T_4), one can calculate the fraction of the tissue [^{125}I]T_3(T_4) that has been locally produced (12), which can also be readily obtained by computing $(1 - R^{-1})$ from the R value shown in Table IV. One can also calculate the mass of T_3 derived from the conversion. The results are shown in the last two columns of Table IV. PTU did not significantly reduce the total or locally generated T_3 in BAT at room temperature. In rats treated with PTU, cold stress resulted in a fivefold greater amount of T_3 locally generated from T_4. Most notably, prazosin abolished the difference.

To estimate the steady-state concentration of T_4 in BAT in euthyroid rats, eight animals were injected with tracer [^{125}I]T_4 and killed either 2 or 4 h after the injection. The results are shown in Table V. The BAT/serum ratio of [^{125}I]T_4 did not change between these times suggesting that equilibrium had been reached by 2 h. For a ratio of 0.078 and a plasma T_4 38 ng/ml (Table I) one can estimate a BAT T_4 concentration of 3 ng/g of tissue, which ranks only behind liver and kidney T_4 content (35) when compared to other tissues.

Discussion

Our earlier studies in hypothyroid adult rats and neonatal rats, both euthyroid and hypothyroid, indicated that in these conditions a large fraction or all the T_3 generated from T_4 in the body was produced via a PTU-insensitive mechanism (3, 19). This was not the only evidence of favoring a type II enzyme as a source of this T_3 because, in addition, we observed that fractional conversion of T_4 to T_3 was maintained or increased in hypothyroidism in spite of the reduced 5'D-I activity in liver and kidney, and that the pretreatment with T_4 or rT_3, at a dosage that reduced the level of 5'D-II, but not that of 5'D-I, resulted in a fall of T_3 production from T_4. Although the brain contains 5'D-II, and the production of T_3 by this tissue may be as high as 17% of that measurable in the plasma pool in neonatal rats (28), this tissue can hardly, if at all, account for those findings. The presence of an enzyme in the BAT that is indistinguishable from the brain and pituitary 5'D-II, prompted us to examine the possibility of this tissue being the source of circulating T_3.

Table V. BAT [^{125}I]T_4 2 and 4 h after Injecting a Tracer Dose of [^{125}I]T_4 to Euthyroid Rats

Time	Tissue content [^{125}I]T_4	BAT/serum [^{125}I]T_4
h	*%/g*	*ml/g*
2	0.35±0.03	0.070±0.008
4	0.43±0.03	0.087±0.011

Eight euthyroid rats weighing 141±8 g (SD) were injected with [^{125}I]T_4 and killed 2 and 4 h after the injection. Serum and tissues were handled as described in Methods. The results have been normalized to 130 g of BW, to make them comparable to those shown in Table IV.

The results just described suggest that, indeed, this tissue, when stimulated to a degree as seen in hypothyroidism, may account for a major fraction of T_3 produced in the exchangeable pool. In addition, the present results show that BAT 5'D, when stimulated, may increase severalfold the content of T_3 in this tissue, which may have implications that will be discussed below.

Our results showed that when animals are chronically exposed to low ambient temperature, there was an increased production of T_4 and T_3. Most relevant for the purpose of these studies, the extrathyroidal T_3 production was about fourfold increased and, further, the PTU-insensitive extrathyroidal T_3 production was augmented approximately 10-fold (Table I). Increased plasma T_3 concentrations or at least increased T_3 to T_4 ratios in circulation in rats exposed to cold have been previously reported (36–38). The relative increment in T_3 has been interpreted as due largely to T_3 enrichment of the thyroidal secretion since this is seen after TSH stimulation and TSH is increased by cold stress (39). More recently, Scammel et al. (40, 41) reported that liver and kidney deiodinase activities were increased in cold-exposed animals, and suggested that the mechanism was the elevated serum thyroxine derived from thyroidal secretion, i.e., that liver and kidney responses reflected the mild degree of hyperthyroidism of the cold-adapted animals. We were unable to reproduce their results because we found no increase in liver or kidney 5'deiodinase in rats exposed to cold for 7 wk (Table II). The explanation for the discrepancy is not readily apparent, although both the experimental protocol and the assay conditions were slightly different. Probably more significant is the fact that, even if those increments described by Scammell et al. (40, 41) were real, they do not seem enough to account for the increased fractional conversion and T_3 production seen in the present studies. If the liver and kidney had been the only source of the excess T_3, the pretreatment with PTU would have markedly reduced the production of T_3 and to a greater extent in the animals exposed to low temperature than in the room temperature controls. Notwithstanding, our results do not exclude the possibility that liver and kidney participated in the increased extrathyroidal T_3 production in that the absolute difference in the rate of T_3 production induced by the administration of PTU was larger in the cold-exposed animals than in the rats maintained at room temperature. Unfortunately, as the PTU-sensitive component of T_3 generation is obtained from the difference between independent observations, the variance is not known and, hence, is not possible to analyze statistically. Be that as it may, our data strongly suggest that BAT is the source of the PTU-insensitive portion of T_3 production in cold-adapted rats. Thus, we know from previous experiments (21) that cold does not increase the activity of the type II enzyme in the brain and the pituitary gland, and on the other hand, we found a marked elevation in the 5'deiodinase activity in the BAT. The increment was more impressive when the augmented BAT mass and protein content are taken into consideration: the quantity of enzyme in the intrascapular BAT was greater than sixfold increased, which is certainly consistent with this tissue being a source of at least part of the increased T_3 production.

To supplement the information provided by the first experiment, we explored in a more specific fashion the potential role of brown fat on T_3 production during cold exposure. Some of the questions emerging from the first experiment were: the thyroidal secretion of T_3, the enhanced liver and kidney 5'D-I activity reported by others in long-term cold exposure (40, 41), and the specificity of BAT 5'D-II as the source of PTU-insensitive

T_3—as opposed to other tissues containing 5'D-II. To deal with these problems, we used thyroidectomized rats, we shortened the cold exposure, and we manipulated specifically BAT 5'D-II with prazosin. In addition, we looked at the BAT T_3 content, because an increment of T_3, if it was demonstrated to be produced locally, would be another feature favoring this tissue as a source of the increased plasma T_3. Finally, the dose of PTU used in these experiments was the highest reported to the best of our knowledge (10 mg/100 g of BW within 16 h) and certainly supramaximal for liver and kidney deiodinase inhibition (7, 13). Notably, under these circumstances, a two- to threefold increase in PTU-insensitive T_3 production was >85% reduced by pretreatment of the animals with prazosin. In addition, cold exposure resulted in a marked elevation of the concentration of T_3 in the BAT, which was not accounted for by the increased plasma concentration and was abolished by treatment with prazosin. Both the findings in the plasma and in the BAT were paralleled by the changes in the BAT 5'deiodinase observed in these animals (Fig. 5), whereas, regardless of the experimental condition, the liver 5'deiodinase was markedly reduced by PTU pretreatment. The results clearly demonstrate that BAT can produce significant amounts of T_3, both for the tissue itself and for the plasma pool.

Although PTU has been one of our main tools to evaluate the origin of T_3 in plasma and tissues (i.e. type I vs. type II T_4 5'deiodinase) in these and in previous studies (3, 13, 19), its use for this purpose in hypothyroxinemic states has recently been criticized on the grounds that the low substrate concentrations pertaining under these circumstances might make the "type I" enzyme less sensitive to PTU (22). This could occur if the postulated sulfenyl iodide complex of the type I enzyme were not available for interaction with PTU. Although we believe that there may be methodologic explanations for the apparent presence of a low K_m, relatively PTU-insensitive, renal enzyme described by Goswami and Rosenberg (22), such arguments are not relevant to the present studies of BAT 5'deiodinase in rats with normal serum T_4 concentrations and typical liver and kidney responses to PTU-induced inhibition of 5'deiodinase. Unlike the renal 5'deiodinase, BAT 5'D-II is stimulated by cold, and its V_{max} is increased, not decreased, in hypothyroidism (20, 21). Unless the enzyme described by Goswami and Rosenberg (22) was increased in hypothyroidism, stimulated by the sympathetic nervous system, sensitive to inhibition by prazosin, and resistant to PTU inhibition under the conditions of the present experiments, the results shown here cannot be explained by that kidney enzyme.

The present findings have several important implications. First, they support the concept that the sympathetic nervous system and thyroid hormone act synergistically in the body to increase calorigenesis under some circumstances, e.g. cold adaptation. Secondly, these results add further support to the hypothesis that BAT may be an important source of T_3 in hypothyroxinemia, as demonstrated by Lum et al. in humans (42), and on the neonatal T_3 surge, situations in which there is abundant BAT and increased sympathetic activity. Our estimate of a BAT T_4 of 3 ng/g (Table V) indicates that this tissue compares in this respect favorably with liver and kidney (43), also putative sources of plasma T_3. It is true that the total body mass of BAT is less than the liver, and that the V_{max} of BAT 5'D is markedly lower than liver and kidney 5'D-I, but the catalytic efficiency of this Type II enzyme could compensate for this difference (14–16).

Lastly, the present findings suggest that thyroid hormone may be important for the function of the BAT and that we should revise the concept that thyroid hormone only plays a permissive role in the function of this tissue as heat dissipator, as suggested by Himms-Hagen (44). We have recently shown that the BAT 5'D is under complex hormonal regulation (45) and, given the wide fluctuations of the enzyme activity, as shown in these and previous studies (19, 21, 45), it is conceivable that the content of T_3 in the BAT changes markedly without being reflected by the serum levels of T_4 or T_3, and vice versa, that marked fluctuations in serum levels of T_4 or T_3 are "buffered" by changes in BAT 5'D. Thus, when the animals are given just physiologic replacement doses of T_4, and the brown fat is stimulated through the sympathetic nervous system, as done by Triandafillou et al. (45), the content of T_3 of this tissue increases, as we have demonstrated in the present studies. Had the increment in BAT 5'D not taken place, the possibility remains open that the calorigenic response of this tissue might have been less. Our results suggest that BAT, which is demonstrable in adult humans under some circumstances (47), could play a role in the pathogenesis of obesity (48) and that alterations in BAT 5'D could contribute to such alterations (49).

Acknowledgments

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