Direct assessment of brown adipose tissue as a site of systemic tri-iodothyronine production in the rat

Jose A. FERNANDEZ, Teresa MAMPEL, Francesc VILLARROYA and Rosario IGLESIAS*

Fisiologia General, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08071-Barcelona, Spain

Tri-iodothyronine (T_3) production by interscapular brown fat was studied by measurements of arterio-venous differences and blood flow across the tissue in rats exposed to the following situations: controls, acute cold, chronic cold and starvation. Results demonstrate that brown adipose tissue is a source of systemic T_3 in the rat and that the T_3 release is modulated according to the physiological situation of the animal: increased in cold exposure and inhibited in starvation.

INTRODUCTION

It is well known that less than 40% of the active thyroid hormone 3,3',5-tri-iodothyronine (T_3) is released directly by the thyroid gland, and that the main source of systemic T_3 is extrathyroidal 5'-deiodination of thyroxine (T_4) (Larsen *et al.*, 1981). Peripheral 5'-deiodination of T_4 is due to the action of an iodothyronine 5'-deiodinase activity that is present in many tissues. Available data point to the existence of at least two enzymic forms, which differ in kinetics, tissue localization and regulation. Type I enzyme is mainly present in liver and kidney, shows Ping-Pong-type kinetics, has K_m values for T_4 and reverse T_3 (rT_3) in the micromolar range and is inhibited uncompetitively by propylthiouracil (Visser, 1979; Leonard & Rosenberg, 1980). Type II enzyme has been found in brain, pituitary and brown adipose tissue; it has sequential-type kinetics, lower K_m for T_4 and rT_3 (nanomolar range), and is not inhibited by propylthiouracil (Visser *et al.*, 1982, 1983; Leonard *et al.*, 1983). The relative contribution of the two activities to circulating T_3 differs from one physiological situation to another. In fact, 30–40% of the T_3 is produced by a propylthiouracil-insensitive pathway (mainly type II enzyme) in adult euthyroid rats (Silva *et al.*, 1982), but it can rise to as much as 70–95% in cold-acclimated (Silva & Larsen, 1985), pre-weaning (Silva & Matthews, 1984) or hypothyroid rats (Silva *et al.*, 1984). Brain and pituitary could hardly contribute to circulating T_3 , as T_3 pools in these tissues seem to interchange very slowly with the plasma pool (Silva *et al.*, 1982). Thus brown adipose tissue is suspected to be a possible source of substantial T_3 generation, especially in situations of predominance of the propylthiouracil-insensitive pathway.

Brown-fat iodothyronine 5'-deiodinase (EC 3.8.1.4) activity has been reported to depend on the sympathetic stimulation of the tissue (Silva & Larsen, 1983) and correlates closely with its thermogenic activity, either in situations of increased thermogenesis, such as cold exposure (Sundin & Cannon, 1980; Silva & Larsen, 1983) and the neonatal period (Sundin & Cannon, 1980; Iglesias *et al.*, 1987), or in those of diminished thermogenic activity, such as lactation (Giralt *et al.*,

1986; Villarroya *et al.*, 1986), starvation (Desautels, 1985; Glick *et al.*, 1985) and genetic obesity (Himms-Hagen & Desautels, 1978; Kates & Himms-Hagen, 1985). Moreover, a good parallelism between brown-adipose-tissue 5'-deiodinase activity and circulating T_3 concentrations has been observed in the above-mentioned situations (Glick *et al.*, 1985; Kates & Himms-Hagen, 1985; Silva & Larsen, 1985; Giralt *et al.*, 1986).

The aim of the present work was to study whether brown fat is an actual site of T_3 production in the rat, by determining arterio-venous T_3 differences together with blood flow across the interscapular brown adipose tissue, and to correlate these data with the iodothyronine 5'-deiodinase activity of this tissue. The study was performed in basal conditions as well as in physiological situations of increased (acute and chronic cold-exposure) and decreased (starvation) metabolic activity of brown fat.

MATERIALS AND METHODS

Animals and experimental groups

Male Wistar rats were fed *ad libitum* on a stock diet (type A04; Panlab, Barcelona, Spain). They were maintained in an automatically controlled light/dark cycle (light 08:00–20:00 h) and temperature (21 ± 1 °C, unless otherwise stated) and were used at 50–60 days of age. Four groups of animals were studied: (1) rats kept at 21 ± 1 °C (controls), (2) rats maintained at 4 °C from 25 days of age until killed (4-week-cold-acclimated), (3) rats kept at 4 °C for 5 h (acute cold-exposed), (4) rats deprived of food for 36 h (starved). Two sets of animals were used: one set was used for measurements of arterio-venous differences of T_3 and T_4 across the interscapular brown fat as well as for the determination of iodothyronine 5'-deiodinase activity in the tissue, and the other set of animals was used for measurements of interscapular brown-adipose-tissue blood flow.

Arterial and venous samples

For the measurements of arterio-venous differences across the interscapular brown adipose tissue, rats were anaesthetized with sodium barbital (50 mg/kg body wt.,

Abbreviations used: T_3 , 3,3',5-tri-iodothyronine; T_4 , thyroxine; rT_3 , 'reverse T_3 ' (3,3',5'-tri-iodothyronine).

* To whom correspondence and reprint requests should be sent.

intraperitoneally). Sulzer's vein, which drains blood flowing through interscapular brown fat, was exposed, a small cut was performed on it and a heparinized capillary was accurately placed on the cut. Blood was allowed to flow directly into the capillary. After collection of a total of 150–200 μ l of blood, haemorrhage was prevented by compression and the skin incision was closed with clips. Then the abdominal cavity was opened and a blood sample (500 μ l) was drawn from the abdominal aorta with an heparinized syringe. The interscapular brown adipose tissue was rapidly dissected out, frozen with solid CO₂ and kept at -70°C until further processing. Blood samples were centrifuged immediately, as haematocrit was determined, and plasma was kept at -30°C until the determination of the hormonal content.

T_4 and T_3 measurements

The concentrations of T_4 and T_3 in plasma were determined by means of radioimmunoassays adapted to measurements in rat plasma (Obregón *et al.*, 1979). The limits of detection were 5 pg of T_4 and 0.7 pg of T_3 ; 1 and 10 μ l of plasma/tube respectively were used for T_4 and T_3 radioimmunoassays. The separation of antibody-bound hormone was achieved through co-precipitation with poly(ethylene glycol) (Carbowax-6000) and bovine γ -globulin (Sigma). All samples were processed in the same assay.

Iodothyronine 5'-deiodinase assay

Fragments of frozen tissue were weighed and homogenized in ice-cold 0.32 M-sucrose/10 mM-Hepes (Sigma)/10 mM-dithiothreitol (Calbiochem), pH 7.0. Iodothyronine 5'-deiodinase activity was assayed by measuring the $^{125}\text{I}^-$ liberated from L-[5'- ^{125}I]rT₃ as described previously (Leonard & Rosenberg, 1980; Leonard *et al.*, 1983): a sample of homogenate corresponding to 200–300 μ g of protein [determined by a modification of the Lowry method (Wang & Smith, 1975)] was added to polystyrene tubes containing 50 μ l of 0.2 M-potassium phosphate buffer, pH 7.0, 200 fmol of L-rT₃ (Henning, Berlin, Germany), 20 fmol of purified L-[5'- ^{125}I]rT₃ (2.2 nCi/fmol), 1.5 μ mol of dithiothreitol and 0.1 μ mol of EDTA. Reaction was stopped 60 min later by adding 50 μ l of ice-cold calf serum (diluted 1:1 with 10 mM-propylthiouracil) and 350 μ l of 10% (w/v) trichloroacetic acid. Iodine was separated from iodothyronines by ion-exchange chromatography on Dowex 50W-X2 (Bio-Rad) columns (Leonard & Rosenberg, 1980). Non-enzymic I^- production was estimated by the incubation of tissue-free medium and served as the blank control. High-specific-radioactivity L-[5'- ^{125}I]rT₃ was obtained by iodination with $^{125}\text{I}^-$ (IMS-30; Amersham) with L-3,3'-di-iodothyronine (Henning) as substrate (Kochupillai & Yallow, 1978).

Measurement of blood flow to interscapular brown adipose tissue

This was done with ^{46}Sc -labelled microspheres (NEN-TRAC; New England Nuclear, Dreieich, Germany; mean diameter 15 μ m), essentially as described by Foster & Frydman (1979), with some modifications (Jones & Williamson, 1984). A polyethylene catheter (internal diameter 0.28 mm, external diameter 0.32 mm) was introduced into the femoral artery. Sc-labelled microspheres (about 300 000, approx. 7.5 μ Ci) were injected

Table 1. Arterio-venous differences of T_3 and T_4 across interscapular brown adipose tissue

Results are means $\pm$ S.E.M. for the numbers of animals shown in parentheses. Values are expressed as ng of T_3 /l and μ g of T_4 /l of plasma. A negative difference implies a release by the tissue. For experimental details see the Materials and methods section. Statistical significances of the differences between control and experimental groups are indicated by: * $P \leq 0.01$; ** $P \leq 0.05$; *** $P \leq 0.001$. Arteriovenous differences that are significantly different from zero are indicated by: † $P \leq 0.05$; †† $P \leq 0.01$.

Treatment of rats	Arterial		Venous (Sulzer's)		Arterio-venous differences	
	T_3	T_4	T_3	T_4	T_3	T_4
Control	769 $\pm$ 31 (12)	44.8 $\pm$ 4.5 (12)	874 $\pm$ 47 (12)	48.9 $\pm$ 3.4 (12)	-105 $\pm$ 36 (12)†	-4.1 $\pm$ 4.7 (12)
Acute cold (5 h, 4 °C)	1120 $\pm$ 50 (8)***	65.1 $\pm$ 7.7 (8)*	1429 $\pm$ 108 (8)***	64.5 $\pm$ 7.3 (8)*	-309 $\pm$ 74 (8)*††	+0.7 $\pm$ 3.6 (8)
Chronic cold (4 weeks, 4 °C)	920 $\pm$ 23 (11)**	38.2 $\pm$ 3.6 (11)	1029 $\pm$ 28 (11)*	42.0 $\pm$ 4.8 (11)	-108 $\pm$ 31 (11)††	-3.9 $\pm$ 2.6 (11)
Starved (36 h)	418 $\pm$ 31 (10)***	20.9 $\pm$ 1.6 (10)***	448 $\pm$ 44 (10)***	21.1 $\pm$ 2.0 (10)***	-30 $\pm$ 36 (10)*	-0.3 $\pm$ 1.1 (10)

Table 2. Blood flow, T_3 production and iodothyronine 5'-deiodinase activity from interscapular brown adipose tissue (IBAT)

Results are means $\pm$ S.E.M. for the numbers of animals shown in parentheses. For experimental details see the Materials and methods section. Statistical significances of the differences between control and experimental groups are: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Treatment of rats	Blood flow (ml/min per total IBAT)	T_3 production (pg/min per total IBAT)	Iodothyronine 5'-deiodinase activity	
			(fmol of I^- /h per mg of protein)	(pmol of I^- /h per total IBAT)
Control	0.20 $\pm$ 0.09 (4)	11.6 $\pm$ 4.0 (12)	71.3 $\pm$ 6.7 (13)	3.1 $\pm$ 0.3 (13)
Acute cold (5 h, 4 °C)	0.70 $\pm$ 0.01 (4)**	119.4 $\pm$ 28.6 (8)***	217.7 $\pm$ 64.7 (7)**	9.5 $\pm$ 3.3 (7)*
Chronic cold (4 weeks, 4 °C)	1.19 $\pm$ 0.11 (4)***	70.7 $\pm$ 20.3 (11)**	337.4 $\pm$ 35.6 (11)***	34.1 $\pm$ 3.0 (11)***
Starved (36 h)	0.08 $\pm$ 0.02 (4)	1.3 $\pm$ 1.6 (10)*	34.6 $\pm$ 4.2 (9)***	1.2 $\pm$ 0.1 (9)***

into the left ventricle of the heart by direct cardiac puncture over a 10 s period. A reference blood sample was drawn from the femoral artery at a rate of 0.6 ml/min from 5 s before until 1 min after completion of the injection of microspheres. Interscapular brown adipose tissue was immediately dissected out, weighed and counted for radioactivity in a γ -counter (Nuclear Chicago). Rates of blood flow were calculated on the basis of the reference blood flow, the radioactivity (c.p.m.) found in the reference blood sample and that detected in the tissue, as described by Jones & Williamson (1984).

Statistics

Student's *t* test was used to test the level of significance for the differences between means, and to test whether arterio-venous differences were significantly different from zero.

RESULTS AND DISCUSSION

The concentrations of T_3 and T_4 in arterial plasma and in plasma of blood flowing from the interscapular brown adipose tissue via Sulzer's vein are shown in Table 1. T_3 and T_4 arterial concentrations were significantly increased in rats exposed to acute cold, whereas only T_3 was significantly increased in cold-acclimated rats as compared with control ones. A significant decrease in arterial T_3 and T_4 was observed after 36 h of starvation. These results are essential in agreement with previous reports on circulating thyroid-hormone concentrations in the above-mentioned physiological situations in the rat (Hefco *et al.*, 1975; Van Hardeveld *et al.*, 1979; Glick *et al.*, 1985). For all the experimental situations studied, the concentrations of both hormones in the Sulzer's vein presented changes that paralleled those observed in arterial plasma.

Arterio-venous differences for T_3 and T_4 are also depicted in Table 1. For T_3 , arterio-venous differences were significantly different from zero in control, acute-cold-exposed and cold-acclimated rats, T_3 concentration in the Sulzer's vein being always higher than in the artery. In starved animals, the arterio-venous difference in plasma T_3 was not significantly different from zero. The arterio-venous differences for T_4 were not significantly different from zero for any of the groups studied. These results provide direct evidence of brown

adipose tissue as an actual source of systemic T_3 in control as well as in acute or chronically cold-exposed rats.

Estimation of interscapular brown-adipose-tissue blood flow in our experimental conditions are presented in Table 2. Cold stress produced a significant rise in the blood flow across the interscapular brown fat, the increase being higher in cold-acclimated animals than in the acutely cold-exposed ones. In contrast, starvation produced a large decrease in brown-adipose-tissue blood flow. These results are basically in agreement with those described in acute and chronically cold-exposed rats (Foster & Frydman, 1979; Foster *et al.*, 1980). No previous data are available about the effects of starvation on brown-adipose-tissue blood flow, even though the present findings are consistent with the overall decrease in brown-fat activity reported in starved animals (Rothwell *et al.*, 1984; Desautels, 1985).

The total release of T_3 by interscapular brown adipose tissue (see Table 2) was calculated from data on the arterio-venous differences in plasma T_3 , the haematocrit values and the blood flow across the interscapular brown fat. Acute cold exposure as well as cold-acclimation produced a significant increase in the T_3 release from the interscapular brown adipose tissue. No significant differences in total T_3 release were observed between cold-acclimated and acute cold-exposed rats, as the higher arterio-venous difference of T_3 in the latter was compensated in part by the higher tissue blood flow in the former.

In the experimental situations studied, the similar pattern of changes of the T_3 output from interscapular brown adipose tissue *in vivo* and of the iodothyronine 5'-deiodinase activity measured in brown-fat homogenates (see Table 2) seems to indicate that iodothyronine 5'-deiodinase in brown adipose tissue is related not only to the possible role of the T_3 generated *in situ* but also to a substantial contribution of this tissue to systemic T_3 that can be modulated according to the physiological situation of the animal. Effectively, the specific activity (fmol/h per mg of protein) as well as the total activity (pmol/h for total interscapular brown fat) of iodothyronine 5'-deiodinase in the tissue are significantly decreased in starved animals and increased in acutely cold-exposed and cold-acclimated ones. The changes in brown-fat iodothyronine 5'-deiodinase activity reported here are in agreement with those previously described in cold-

acclimated (Silva & Larsen, 1985) and starved rats (Glick *et al.*, 1985). However, no previous results are available comparing values of brown-fat iodothyronine 5'-deiodinase activity in acute-cold-exposed and chronically cold-acclimated rats. The present results show a significant increase ($P \leq 0.001$) in iodothyronine 5'-deiodinase activity, expressed as pmol/h per total interscapular brown fat, in cold-acclimated rats when compared with acutely cold-exposed ones. This difference contrasts with the similar T_3 release from interscapular brown adipose tissue found in these two situations. This fact can be related to an increase in the availability of substrates *in vivo* (T_4 and/or reduced co-substrate) for 5'-deiodination, leading to T_3 formation in the acute-cold-exposed group and/or to an increase in the utilization by the tissue of the T_3 generated *in situ* in the cold-acclimated group.

The present results on T_3 production help us to approach the estimation of the daily T_3 production by the whole brown fat in the rat, assuming that the interscapular mass accounts for around 20% of the total brown fat and that its activity is on average that in other brown-adipose-tissue bodies (Foster *et al.*, 1980). On these bases, total daily brown-fat T_3 production can be estimated at about 31 and 233 ng/100 g body wt. in control and cold-acclimated rats respectively, and it can be as high as 312 ng/100 g body wt. in acutely cold-exposed animals. This production represents respectively 30 and 60% of the estimated overall T_3 production in the two first-mentioned situations, and it is fully coincident with reports of the propylthiouracil-insensitive T_3 production rate, based on thyroid-hormone turnover studies (Silva & Larsen, 1985). Therefore we can conclude that brown adipose tissue is in fact the main tissue responsible for the peripheral T_3 production via a propylthiouracil-insensitive pathway in the rat and that its rate of T_3 release is highly sensitive to modulation, depending on the physiological status of the animal.

We thank John Mackay for editorial assistance.

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Received 17 November 1986/13 January 1987; accepted 26 January 1987