Iodothyronine 5'-deiodinase activity and thyroid hormone content in brown adipose tissue during the breeding cycle of the rat

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Brown adipose tissue iodothyronine 5'-deiodinase activity is significantly lower in 17-day pregnant rats compared with virgin controls and remains low during late pregnancy and lactation. It fully recovers with abrupt weaning, but only partially with spontaneous weaning. Even though this profile of changes is remarkably in step with the known pattern of modifications in brown fat thermogenesis during the breeding cycle, the lowered iodothyronine 5'-deiodinase activity appearing between days 15 and 17 of pregnancy occurs earlier than the reduction in brown adipose tissue thermogenesis. Brown fat 3,3',5-tri-iodothyronine content is also reduced in late pregnant, early and mid-lactating rats, most probably as a consequence of the lowered 5'-deiodination of thyroxine *in situ*. Acute insulin treatment increases brown fat iodothyronine 5'-deiodinase activity in virgin animals as well as in late-pregnant and lactating rats, despite the lowered basal enzyme activity levels in the latter groups. Thus an impaired response to insulin in brown fat does not appear to be a factor leading to the lowered iodothyronine 5'-deiodinase activity during late pregnancy and lactation.

INTRODUCTION

Brown adipose tissue is considered to be the main site for non-shivering thermogenesis in small mammals. The thermogenic activity of this tissue relies on a mitochondrial proton conductance pathway generated by the uncoupling protein, a specific component of brown fat mitochondria (Nicholls *et al.*, 1986). More recently, a novel feature of brown fat in rodents was described, namely the presence of a type II iodothyronine 5'-deiodinase activity that is highly stimulated by cold exposure or noradrenaline treatment (Silva & Larsen, 1983). Brown fat containing this enzyme activity is an important site for a local (Silva & Larsen, 1985) and systemic 3,3',5-tri-iodothyronine (T_3) production, especially in situations such as cold exposure when the tissue is especially active (Fernández *et al.*, 1987). Moreover, the parallel changes in brown fat thermogenic and iodothyronine 5'-deiodinase activities under different physiological or experimental conditions suggest that changes in the tissue T_3 content, generated by thyroxine (T_4) deiodination *in situ*, may be important for the thermogenic activity of the tissue (Kates & Himms-Hagen, 1985; Silva & Larsen, 1985; Bianco & Silva, 1987).

Brown adipose tissue thermogenic activity is substantially modified during the breeding cycle of the rodents. In late pregnancy and especially during lactation there is a progressive decrease in brown fat thermogenic capacity, characterized by an important hypotrophy of the mitochondrial component of the tissue as well as a decrease in the specific mitochondrial proton conductance pathway activity (Trayhurn *et al.*, 1982; Villarroya *et al.*, 1986). Recently, we have reported that late-pregnant and mid-lactating rats also show a reduced

basal and noradrenaline-induced iodothyronine 5'-deiodinase activity in brown fat (Giralt *et al.*, 1986).

This aim of the present work was to establish fully the profile of changes in brown fat iodothyronine 5'-deiodinase during the breeding cycle of the rat (pregnancy, lactation, and weaning), as well as to determine whether there are changes in the actual thyroid hormone content of the tissue associated with the 5'-deiodinase modifications. By taking into account the recent report that insulin is a powerful stimulator of brown fat iodothyronine 5'-deiodinase (Silva & Larsen, 1986), as well as the impaired metabolic effects of insulin in brown fat from late pregnant and lactating rats (Ferré *et al.*, 1986), the study was extended to determine the effects of insulin on the iodothyronine 5'-deiodinase activity in brown adipose tissue at different stages of the rat's breeding cycle.

MATERIALS AND METHODS

Animals

Female Wistar rats were maintained in an automatically controlled environment ($21 \pm 1^\circ\text{C}$; 12 h-light/dark cycle). They were fed *ad libitum* a stock diet (A-03 type; Panlab, Barcelona, Spain) and were studied in different times of pregnancy and lactation as indicated below. Female rats weighing 175–200 g were mated with adult males. The day of pregnancy was determined by the presence of spermatozoa in vaginal smears. When lactating rats were studied, litter sizes were adjusted to 9–11 pups at birth. Sudden weaning was induced in 15-day-lactating rats by removing pups 24 h prior to sacrifice. When the effect of acute insulin administration

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

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was studied the animals were sacrificed 3 h after the injection of saline or insulin (1 unit/100 g body weight; Actrapid monocomponent; Novo) into the tail vein.

Rats were sacrificed by decapitation and the interscapular brown adipose tissue was rapidly dissected, frozen with solid CO₂ and kept at -70 °C until further processing.

Iodothyronine 5'-deiodinase assay

Fragment of frozen tissue were weighed and homogenized in ice-cold 0.32 M-sucrose/10 mM-Hepes/10 mM-dithiothreitol (DTT), pH 7.0. Homogenates were centrifuged at 80 g for 10 min, fat supernatants were discarded and the infranatants were used to determine iodothyronine 5'-deiodinase activity. Protein content was determined by a modification of the Lowry method (Wang & Smith, 1975). Outer ring (5') deiodinating activity was assayed by quantifying the ¹²⁵I⁻ liberated from L-[5'-¹²⁵I]rT₃ as described previously (Leonard & Rosenberg, 1980; Leonard *et al.*, 1983). Protein (200–300 µg) was added to polystyrene tubes containing 50 µl of an incubation medium consisting of 4 nM-rT₃, 0.4 nM purified L-[5'-¹²⁵I]rT₃ (500 c.p.m./fmol), 30 mM-DTT, 0.2 M-potassium phosphate buffer (pH 7.0)/2 mM-EDTA. Incubations were performed on a shaking bath at 37 °C under a N₂ atmosphere for 60 min. Reaction was stopped by adding 50 µl of ice-cold diluted calf serum (1:1, calf serum/10 mM-propylthiouracil) and 350 µl of 10% (w/v) trichloroacetic acid. Separation of iodine from iodo-

thyronines was done by ion-exchange chromatography on Dowex 50W-X2 column (Bio-Rad) equilibrated in 10% (v/v) acetic acid (Leonard & Rosenberg, 1980). Assays were performed in triplicate. Non-enzymic I⁻ production was estimated by the incubation of tissue-free medium which served as the blank control.

Tissue T₄ and T₃ determinations

Extraction of iodothyronines from brown adipose tissue was carried out as described previously (Morreale de Escobar *et al.*, 1985). Briefly, frozen tissue was weighed, homogenized in 1 mM-propylthiouracil/methanol and extracted with chloroform/methanol (2:1, v/v). Iodothyronines were back-extracted from chloroform/methanol extracts with 0.05% CaCl₂ and then concentrated and further purified with Bio-Rad AG 1X2 resin columns. After a series of washes (Morreale de Escobar *et al.*, 1985), which were discarded, iodothyronines were eluted in 70% (v/v) acetic acid. Individual recoveries of extracted iodothyronines were assessed by adding 1500–2000 c.p.m. of L-[5'-¹²⁵I]T₃ and L-[3',5'-¹³¹I]T₄ to the homogenate as internal tracers. Recoveries usually ranged from 50–75% for [¹²⁵I]T₃ and 40–60% for [¹³¹I]T₄.

Acetic acid eluates were evaporated to dryness and taken up in the radioimmunoassay buffer. The T₃ and T₄ radioimmunoassay were carried out as described elsewhere (Obregon *et al.*, 1981). Samples were assayed at two dilutions and in triplicate at each dilution. When

Fig. 1. Changes in interscapular brown adipose tissue iodothyronine 5'-deiodinase activity during pregnancy and lactation in the rat

Results are means ± S.E.M. (indicated by bars) for 5–10 rats per group. ● Indicates 24 h after abrupt weaning of 15-day lactating rats. Statistical comparisons between virgin controls and experimental groups are shown by: * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

tissue fat-free weight data were used to express thyroid hormone content, lipids were quantified gravimetrically after extraction (Folch *et al.*, 1957) from frozen tissue fragments.

Synthesis of labelled tracers

High specific activity L-[5'-¹²⁵I]rT₃ and L-[5'-¹²⁵I]T₃ were obtained by radioiodination with ¹²⁵I⁻ (IMS-30; Amersham) using as substrate 3,3'-di-iodothyronine and 3,5-di-iodothyronine respectively. The doubly labelled high specific activity L-[3',5'-¹³¹I]T₄ was obtained from L-3,5-di-iodothyronine by labelling with ¹³¹I⁻ (IBS-30; Amersham) (Kochupillai & Yallow, 1978).

Statistics

Statistical comparisons between groups were performed by means of Student's *t* test.

RESULTS AND DISCUSSION

Fig. 1 shows the profile of changes in iodothyronine 5'-deiodinase activity in interscapular brown adipose tissue during pregnancy and lactation in the rat. The enzyme activity, expressed as fmol of I⁻ produced/h per mg of protein, is slightly reduced during the first days of pregnancy to be analysed (days 10 and 15 of gestation) even though the values are not statistically different from virgin control ones. Between day 15 and 17 there is a sudden drop in brown fat iodothyronine 5'-deiodinase activity that attains levels significantly lower than those from virgin controls. The brown fat iodothyronine 5'-deiodinase activity remains significantly reduced during late pregnancy and throughout all the days of lactation studied. These findings agree with our previous report on reduced enzyme activity in brown fat of late-pregnant and mid-lactating rats (Giralt *et al.*, 1986). On day 30 of lactation, when the pups' milk intake is practically zero (Redman & Swenwy, 1976), iodothyronine 5'-deiodinase activity levels show a slight recovery and are significantly (*P* < 0.01) higher than those from 15-day-lactating rats even though the activity is still significantly lower than in virgin rats. Thus it seems that at this early stage of spontaneous weaning the brown fat 5'-deiodinase activity levels are not yet fully normalized. However, as also shown in Fig. 1, mothers abruptly weaned on day 15 of lactation show levels of iodothyronine 5'-deiodinase activity in brown adipose tissue equal to those found in virgin controls.

A close parallel is found between the profile of changes occurring in thermogenic activity in brown fat iodothyronine 5'-deiodinase activity in pregnant and lactating rats, and in spontaneous or abruptly weaned mothers (Isler *et al.*, 1984; Villarroya *et al.*, 1986). The only important exception to this parallel occurs between days 17 and 20 of pregnancy when the thermogenic activity of brown fat is still unchanged (Villarroya *et al.*, 1986), whereas the iodothyronine 5'-deiodinase activity is already greatly diminished. The early lowering of iodothyronine 5'-deiodinase activity during the breeding cycle suggests a possible role for this enzyme, and presumably for the level of T₃ generation *in situ*, in the main adaptation of brown fat to low thermogenic activity during lactation, i.e. reduced mitochondrial content and lowered proton conductance pathway activity.

In a tissue such as brown fat, which possesses a type II iodothyronine 5'-deiodinase activity, it has been claimed

Table 1. Weight, T₃, T₄ and T₃/T₄ ratio of interscapular brown adipose tissue from virgin, 20-day-pregnant and from 5- and 15-day-lactating rats

	Weight (g)	T ₄		T ₃		T ₃ /T ₄ (%)
		(ng/IBAT)	(ng/g of FFW)	(ng/IBAT)	(ng/g of FFW)	
Virgin	0.34 ± 0.01	1.50 ± 0.13	7.96 ± 0.72	0.83 ± 0.08	4.42 ± 0.40	56 × 5.04 (6)
20-Day-pregnant	0.35 ± 0.01	0.88 ± 0.05**	6.45 ± 0.71	0.48 ± 0.05	3.54 ± 0.39	55 ± 5.00 (4)
5-Day-lactating	0.26 ± 0.02**	0.98 ± 0.05*	7.94 ± 0.97	0.34 ± 0.04**	2.94 ± 0.32*	35 ± 3.85* (4)
15-Day-lactating	0.23 ± 0.01***	0.79 ± 0.03**	7.27 ± 0.29	0.21 ± 0.06***	2.11 ± 0.06***	27 ± 1.08** (4)

Results are means ± S.E.M. for the number of animals shown in parentheses. T₃ and T₄ contents are expressed per whole interscapular brown adipose tissue (IBAT) and per gram of fat-free weight (FFW). The T₃/T₄ ratio is expressed as a percentage of the T₄ values. Statistical significance of the differences between virgin controls and the experimental groups are shown by: * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

Table 2. Effects of insulin on iodothyronine 5'-deiodinase activity in interscapular brown adipose tissue from virgin, 20-day-pregnant and 15- and 30-day-lactating rats

Results are means $\pm$ S.E.M. for the number of animals shown in parentheses. Insulin (1 unit/100 g body weight) or saline (controls) were given intravenously 3 h before sacrifice. Statistical significance of the differences between control and insulin-treated rats are shown by *** $P < 0.001$.

	Iodothyronine 5'-deiodinase activity (fmols of I- /h per mg of protein)	
	Control	Insulin
Virgin	24.9 $\pm$ 3.2 (6)	90.6 $\pm$ 16.5 (4)***
20-Day-pregnant	5.6 $\pm$ 0.8 (5)	97.8 $\pm$ 15.5 (4)***
15-Day-lactating	2.8 $\pm$ 0.9 (4)	63.9 $\pm$ 2.5 (4)***
30-Day-lactating	5.6 $\pm$ 0.9 (5)	52.2 $\pm$ 5.1 (4)***

that the T_3 content of the tissue is influenced by the level of 5'-deiodination of T_4 *in situ* (Silva & Larsen, 1985). In fact, the importance of the changes in brown fat iodothyronine 5'-deiodinase activity on the T_3 output from the tissue in different physiological situations has been directly demonstrated (Fernández *et al.*, 1987), although modifications in the enzyme activity that can influence the actual brown fat T_3 content have not until very recently been directly assessed.

We have determined the interscapular brown adipose tissue T_4 and T_3 contents in 20-day-pregnant and in 5-day- and 15-day-lactating and virgin control rats and results are shown in Table 1. By taking into account the substantial changes in brown fat weight and lipid content during pregnancy and lactation (Villarroya *et al.*, 1986), the data have been expressed per whole interscapular brown adipose tissue as well as per gram of fat-free tissue weight. Whole tissue T_4 and T_3 contents were significantly lower in groups when compared with the respective control values. Minimal levels were observed in 15-day-lactating rats (T_4 and T_3 contents were 52% and 25% respectively of the control levels). When expressed per gram of fat-free weight, T_4 concentration in brown fat was unchanged in 20-day-pregnant and in 5-day- and 15-day-lactating rats. T_3 concentration per gram of fat-free weight was not significantly modified in interscapular brown fat from 20-day-pregnant rats, whereas it was significantly reduced in 5-day- and 15-day-lactating rats (66% and 47% of the control values respectively).

The present results are among the first reports of a direct assessment of thyroid hormone content in brown fat using a previously described extraction procedure (Morreale de Escobar *et al.*, 1985). The levels of brown fat T_4 and T_3 found here in control animals are in the range, but slightly higher than those reported by other authors using indirect isotopic techniques (Silva & Larsen, 1985; Van Doorn *et al.*, 1985).

Present data on parallel decreases in brown adipose tissue iodothyronine 5'-deiodinase activity and thyroid hormone content during pregnancy and lactation support the importance of deiodination *in situ* on the actual brown fat T_3 . Moreover, during the foetal period, a close correlation between increased iodothyronine 5'-de-

iodinase and high T_3 content in brown adipose tissue has also been described recently (Ruiz de Oña *et al.*, 1987). Thus changes in brown fat T_4 5'-deiodination in different physiological conditions due to a modified iodothyronine 5'-deiodinase activity seem to result not only in changes in the T_3 output from the tissue but also in its actual T_3 content.

On the other hand, the fact that the decrease in brown fat thermogenic activity during lactation is associated with a parallel decrease not only in the 5'-deiodinating activity but also in the actual T_3 content of the tissue reinforces the idea that this hormone may play an important role in the thermogenic activity of the tissue, perhaps by modulating the level of uncoupling protein gene expression, as suggested by Bianco & Silva (1987), or by some as yet unknown mechanism.

In considering the possible regulatory factors responsible for the changes observed in brown fat iodothyronine 5'-deiodinase during the breeding cycle, it should be said that this enzyme activity in brown fat is known to be modulated by the sympathetic activity acting upon the tissue through an α_1 -adrenergic mechanism (Silva & Larsen, 1983). Thus the reduction of sympathetic activity occurring in brown fat in mid-lactating rats (Villarroya *et al.*, 1987) would be expected to reduce 5'-deiodinase activity in brown adipose tissue. However, as previously shown, a marked decrease in iodothyronine 5'-deiodinase activity has already occurred by late pregnancy when the sympathetic activity acting upon the tissue is unlikely to be substantially depressed. Recently, Silva & Larsen (1986) reported that insulin is a powerful stimulator of iodothyronine 5'-deiodinase through a direct mechanism independent of sympathetic activity and requiring protein synthesis. It is well known that brown fat is highly sensitive to insulin action, which stimulates different metabolic events such as glucose uptake or lipogenesis (Czech *et al.*, 1974; McCormack & Denton, 1977). It has also been reported that brown fat is a target site of insulin resistance in late pregnancy and lactation (Ferré *et al.*, 1986). Therefore, we investigated the effects of a single dose of insulin on brown adipose tissue iodothyronine 5'-deiodinase activity in virgin, 20-day-pregnant and in 15-day- and 30-day-lactating rats to assess whether the insulin action on this enzyme activity is different in the various physiological conditions.

The results are presented in Table 2 and indicate that after an intravenous single dose of insulin there is a 4-fold increase of brown fat iodothyronine 5'-deiodinase activity in virgin control rats. Despite the low basal 5'-deiodinase activity levels in brown fat from 20-day pregnant and from 15-day- and 30-day-lactating rats, acute levels of insulin cause a % increase in the enzyme activity in the three groups even higher than that occurring in virgin animals, although only in 20-day-pregnant rats were the levels of iodothyronine 5'-deiodinase activity achieved similar to those obtained in insulin-stimulated virgin animals. Therefore, even though the low insulin levels during lactation (Flint *et al.*, 1979) may contribute to the lowering of brown fat iodothyronine 5'-deiodinase activity, impaired insulin sensitivity in this tissue cannot be claimed as a factor leading to reduced enzyme activity during pregnancy and lactation. In particular, this is because high circulating insulin levels (Herrera *et al.*, 1969) are associated with unchanged insulin-induced 5'-deiodinase activity in 20-day pregnant rats. In summary, other regulatory mech-

anisms need to be researched to understand the reduction brown fat iodothyronine 5'-deiodinase activity during the breeding cycle and especially during mid pregnancy. Although chronic progesterone treatment of virgin rats does lead to any reduction in brown fat 5'-deiodinase activity (O. Viñas, M. Girat, R. Iglesias, F. Villarroya & T. Mampel, unpublished work), the possible influence of hormones, specifically those involved in the reproductive cycle, probably deserves special attention.

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