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EVIDENCE FOR A DIFFERENTIAL PHYSIOLOGICAL MODULATION OF BROWN FAT IODOTHYRONINE 5'-DEIODINASE ACTIVITY IN THE PERINATAL PERIOD

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SUMMARY: Brown adipose tissue iodothyronine 5'-deiodinase increases progressively in fetuses from the day 17 of pregnancy on, it reaches peak values on the 20th day of gestation and declines in the last days of fetal life as well as during the first day of life. Birth of premature fetuses causes a sudden drop in the enzyme activity. Postmaturity is associated to a decrease in brown fat 5'-deiodinase similar to that found after birth in fetuses born at term. In the first hours of life brown fat iodothyronine 5'-deiodinase is essentially insensitive to the cold-stimulus. Present data indicates that, differently from adult rats, brown fat iodothyronine 5'-deiodinase activity during the perinatal period is dissociated from the thermogenic activity of the tissue. It is suggested that factors different from the action of the sympathetic nervous system may play a main role in brown fat iodothyronine 5'-deiodinase activity modulation in the fetal and neonatal life.

INTRODUCTION: Since iodothyronine 5'-deiodinase activity was first described in brown adipose tissue a few years ago (1,2), an active research has been developed on its physiological significance and regulation. Similarly to what occurs with the thermogenic activity of the tissue, brown fat iodothyronine 5'-deiodinase in the adult rat is mainly modulated by the action of the noradrenaline coming from the sympathetic innervation to the tissue (2,3). This fact explains the concurrence between brown fat thermogenesis and iodothyronine 5'-deiodinase activity in different physiological situations (4,5,6) and it has been proposed that "in situ" T_3 generation, thanks to the 5'-deiodination of T_4, may be an important event related to the biochemical mechanisms of brown fat thermogenesis and specially to the modulation of uncoupling protein gene expression (7).

Brown fat thermogenesis is specially active during the neonatal period (8) .When we previously studied the changes in iodothyronine 5'-

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deiodinase activity in rat brown adipose tissue during development, an increase in the enzyme activity was found a few days after birth with respect to the immediately postnatal levels (9). This neonatal increase was closely parallel with the augmented brown fat thermogenesis in this period (10). However, values of brown fat iodothyronine 5'-deiodinase in fetuses two days before birth were found to be extremely high, much more than those of the postnatal peak and in the range of the values corresponding to maximally cold-stimulated brown fat iodothyronine 5'-deiodinase in adult rats (9). This was a surprising finding as fetal brown fat does not show a high thermogenic activity nor it is subjected to physiological stimulus, such as cold environment, that stimulate the enzyme activity in adult rodents (8). Thus, fetal life appeared to be probably the first physiological, not-pathological, situation when a dissociation between brown fat thermogenesis and iodothyronine 5'-deiodinase activity was found.

The aim of the present work has been to study the main physiological modulation of iodothyronine 5'-deiodinase in the perinatal period, in order to assess its differential features with respect to the adult animals. The sequential changes in brown fat iodothyronine 5'-deiodinase activity during the fetal and a few hours postnatal life of the rat have been determined. The role of birth, pre- and post-maturity, and postnatal environment temperature have been also studied.

METHODS: Female Wistar rats (200-210 g b.w.) were mated with adult males and the day of pregnancy was determined by the presence of spermatozoals in vaginal smears. They were fed with a stock diet (A03 type, Panlab, Spain) and kept in a controlled environment (12 h light-dark cycles), environment temperature being 21 ºC unless otherwise stated. When the profile of prenatal changes in brown fat iodothyronine 5'-deiodinase activity was studied, caesarian sections of pregnant rats in the day 17, 18, 19, 20, 21 or 21.5 of gestation were performed in a thermostatized chamber (37 ºC). Fetuses were extracted and the interscapular brown adipose tissues of each litter were removed and pooled . When the postnatal period was studied, pups remained with their mother after spontaneous delivery and they were sacrificed at 0 (considered as when all pups are born but they have not still initiated suckling) 2, 6, 12 and 24 hours after birth. For studies on the effects of postnatal environment temperature, fetuses were extracted by caesarian section in the thermostatized chamber (37 ºC). Two-three pups were sacrificed immediately (0 hours) and half the remaining litter was placed at 21 ºC whereas the other half was kept at 37ºC. This study was performed in at-term (day 21.5 of pregnancy) premature (day 20.5 of pregnancy) and postmature (day 22.5 in pregnant rats treated daily with 7 mg of subcutaneous progesterone from the day 20 of pregnancy, on) fetuses. Pups were sacrificed 2, 12 and 24 hours after birth, with the exception of the postmature group that was studied only at 2 and 12 hours of extrauterine life. In all the cases, fetuses or pups were killed by beheading, interscapular brown adipose tissue was extracted, immediately frozen in dry-ice and kept at -80ºC until further processing. Frozen brown adipose tissue samples were weighed and homogenized in ice cold

0.32 M sucrose, 10 mM HEPES (Sigma), 10 mM DTT (Calbiochem), pH 7.0. Homogenates were centrifuged at 80 x g for 10 min, the fat supernatants were discarded and the infranatants were used for the iodothyronine 5'-deiodinase assay. The protein content was determined as already reported (11). Outer-ring (5') deiodinating activity was assayed by quantifying the $^{125}I^-$ liberated from L-(5'-^{125}I)-rT_3 as previously described (1,12). The enzymatic activity was assayed at a final concentration of 2 nM rT_3, 0.2 nM purified L-(5'-$^{129}I^-$)-rT_3 (5000 cpm/fmols), 20 mM DTT, 0.1 M potassium phosphate buffer (pH 7.0), 1 mM EDTA. Incubations had a final volume of 100 µl containing 0.2-0.3 mg of infranatant protein and they were performed on a shaking bath, at 37 ºC under nitrogen atmosphere during 60 min and were stopped by adding 50 µl ice-cold diluted calf serum/10 mM propylthiouracil (1:1) and 350 µl 10% trichloroactetic acid. Separation of iodine from iodothyronines was done by ion-exchange chromatography on Dowex-50W-X2 (Biorad) columns equilibrated in 10% acetic acid (12). Assays were performed in triplicate. Non enzymatic I^- production was assessed by incubations of free tissue medium and used as blank control. High specific activity L-(5'-^{125}I)-rT_3 was obtained by radio-iodination with $^{125}I^-$(IMS-30, Amersham) using 3,3'-diiodothyronine as substrate (13). Statistical significance of comparisons between groups were performed by means of the Student's t test.

RESULTS AND DISCUSSION: The pattern of pre- and immediately postnatal levels of iodothyronine 5'-deiodinase activity in rat brown adipose tissue is depicted in Fig. 1. Brown fat iodothyronine 5'-deiodinase activity is already detectable in the day 17 of the fetal life, it increases progressively from the day 18 on and reaches its maximum in fetuses at the day 20 of gestation. The 5'-deiodinase activity declines

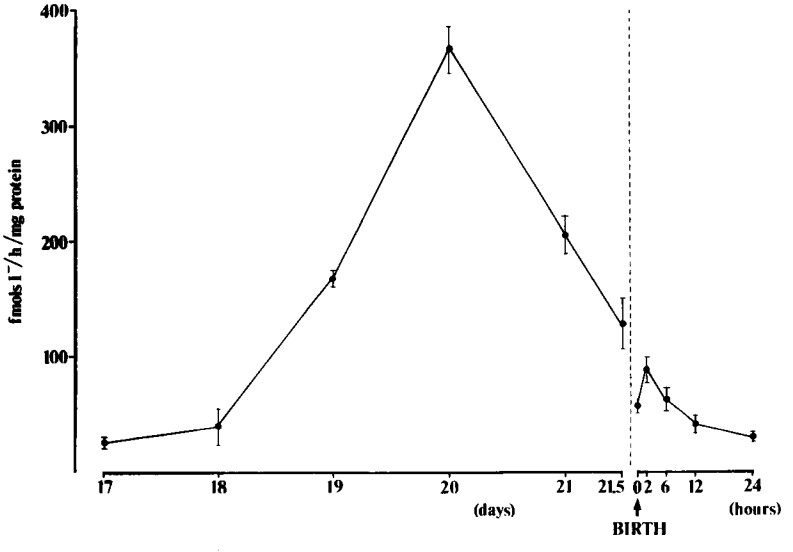

Figure 1
Iodothyronine 5'-deiodinase activity (fmols I^-/h/mg protein) in rat brown adipose tissue during the fetal and immediately postnatal life. Each point represents the means ± SEM of 5-8 litters of the age indicated. For each litter individual fetal or neonatal brown adipose tissues had been pooled.

during the last days of the fetal life and pups at birth show enzyme activity levels similar to those found in fetuses from 18-day pregnant rats. Brown fat iodothyronine 5'-deiodinase activity continues to decrease throughout the first day of life. The peak values of 5'-deiodinase activity in the day 20 of gestation are quantitatively important, in the range of those reported for adult rats in situations of maximally stimulated brown fat 5'-deiodinase activity, such as cold exposure (2,5).

Table 1 summarizes the effects of birth and environment temperature on brown fat iodothyronine 5'-deiodinase activity. Caesarian sections of fetuses in the day 20.5 of gestation (premature birth) cause a sudden drop in brown fat 5'-deiodinase activity which attain, after two hours of extrauterine life, levels similar than those found in two-hour old pups born at-term. Postmature fetuses (day 22.5 of gestation in progesterone-treated mothers) show a lowered iodothyronine 5'-deiodinase activity when compared with at-term fetuses whereas their enzyme activity levels are not significantly different from 24 hours-old pups born at-term. Fetuses from progesterone-treated mothers sacrificed at term showed levels of 5'-deiodinase activity (115 ± 14 fmols I^-/h/mg

Table 1

Iodothyronine 5'-deiodinase activity in brown adipose tissue from rat pups different hours after caesarian section. Effect of gestational age and environment temperature

Hours after caesarian section		Premature	At-term	Postmature
0		226 ± 35 +	122 ± 17 +	51 ± 6 +
2	37ºC	71 ± 14	72 ± 7	40 ± 8 +
	21ºC	70 ± 6	54 ± 8	36 ± 3
12	37ºC	35 ± 5	48 ± 10	67 ± 13
	21ºC	74 ± 16	79 ± 11	80 ± 8
24	37ºC	23 ± 7	28 ± 8	-
	21ºC	46 ± 11	49 ± 5 *	-

For experimental details see Methods section. Results are expressed as fmols I^-/h/mg protein. They are means ± SEM of samples corresponding to 5-8 litters. Statistical significance ($p < 0.05$) of comparisons between pups of the same postnatal age but maintained at different environment temperatures is shown by *. Statistical significance ($p < 0.05$) of comparisons between premature or postmature pups of the same age and environment temperature, and at-term pups is shown by +

protein) that were not significantly different from those in fetuses from untreated dams. Concerning the effects of environment temperature in neonatal brown fat 5'-deiodinase, data in Table 1 indicate that a substantial cold-stress (21ºC) during the first day of extrauterine life does not cause important changes in the brown fat 5'-deiodinase activity of neonates born at-term nor in premature or postmature fetuses, when compared with pups maintained at 37ºC. Only 24 hours after birth, pups born at-term and kept at 21 ºC showed a significant but slight increase in the enzyme activity.The profile of decay in brown fat 5'-deiodinase during the first day of extrauterine life in at-term fetuses extracted by caesarian section was essentially the same than the observed in spontaneously born pups (see Fig 1). These last data can be summarized as that birth appears not to be necessary for the decrease in brown adipose tissue 5'-deiodinase that occurs perinatally but, if birth occurs in a moment of intrauternine life when brown fat 5'-deiodinase is high, it causes a sudden decrease in the enzyme activity.

The overall results presented here on brown fat 5'-deiodinase in the pre- and immediately postnatal life of the rat point to a differential behavior of this enzyme activity in the perinatal period when compared with the adult, specially when its relationship with the thermogenic activity of the tissue is considered. Thus, a very high 5'-deiodinase activity occurs in the fetal life when thermogenesis is not activated. whereas after birth, when the mechanisms of brown fat thermogenesis and specially the uncoupling protein gene expression are rapid and highly increased (14), iodothyronine 5'-deiodinase activity declines progressively. Furthermore iodothyronine 5'-deiodinase in brown fat appears to be considerably insensitive to the cold stimulus in the first hours of life, differently from what occurs in adults and even in 15-day old pups (9).

This characteristic behavior of iodothyronine 5'-deiodinase activity in brown fat during the pre and immediately postnatal life raises the question of which can be the mechanisms that induce such a profile of enzyme activity changes during the perinatal period. First it is considered that the sympathetic nervous system innervating brown adipose tissue is not fully developed neither in the fetal life nor in the first hours after birth (8). Thus, noradrenaline, the main physiological modulator of brown fat 5'-deiodinase in adult rats (4) would not be probably the responsable for the changes in the enzyme activity reported here and specially for the high prenatal activity levels. Among the other hormonal factors known to influence brown fat 5'-deidoinase activity ,the recent report of insulin as a powerful stimulator of brown fat iodothyronine 5'-deiodinase in adult rats (15) probably needs

special atention. The pattern of changes of fetal and neonatal brown fat 5'-deiodinase levels, the ability of premature birth to cause a sudden decline in the enzyme activity and the lowered brown fat iodothyronine 5'-deiodinase in postmature fetuses are in a striking good parallelism with the known changes in circulating insulin levels in these situations (16,17). Thus, further research will be necessary to elucidate if insulin action would play a main role in the modulation of fetal and neonatal brown fat iodothyronine 5'-deiodinase activity.

Concerning the possible functional role of brown fat iodothyronine 5'-deiodinase activity in the periods studied here, the lack of concurrence between thermogenic and 5'-deiodinase activities seems to exclude the possibility of a direct role of the locally produced T_3 in the modulation of the degree of uncoupling protein gene expression during the perinatal period, differently from what has been suggested for adult animals (7). However,the question of the possible functional role of the prenatal peak in the enzyme activity remains to be solved. Recently it has been proposed that a high fetal "in situ" T_3 generation may be involved in the prenatal differentiation of brown adipose tissue and specially in the induction of the uncoupling protein gene expression. This proposal has been formulated from the comparison between the profile of iodothyronine 5'-deiodinase activity and the sequential appearance of the mRNAs corresponding to mitochondrial proteins in the prenatal development of brown fat in bovines (Giralt et al. manuscript sent for publication). Our present data suggest that this hypothesis may be extended to the prenatal development of brown fat in rodents, that would be good models for future research on the relationships between prenatal iodothyronine 5'-deiodinase activity and brown fat differentiation.

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