Retinoid X receptors (RXRs) and T_3 receptors (T_3Rs) mRNA expression during rat brown adipose tissue development

Expresión de mRNA de receptores de retinoide X (RXRS) y receptores de T_3 (T_3Rs) durante el desarrollo del tejido adiposo marrón en la rata

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ABSTRACT

T_3 is a transcriptional activator of the brown adipose tissue (BAT) uncoupling protein gene (*ucp*). Retinoid X receptor (RXR) is the heterodimeric partner of T_3 receptor (T_3R) in the T_3 response element (TRE) of thyroid hormone target genes. Present study demonstrates that RXRα, RXRβ and RXRγ genes are expressed in the developing BAT. RXRα mRNA expression was high in BAT from 18-day-old fetuses and decreased progressively during development, whereas RXRβ mRNA levels were unmodified. mRNA levels of RXRγ increased sharply between days 18 and 20 of fetal life, in concurrence with the onset of *ucp* gene transcription. Concomitantly, T_3R α and β_1 mRNA levels were lower in 18-day-old than in 20-day-old fetuses. Our results are compatible with the involvement of RXRs in the regulation of *ucp* gene transcription by T_3 during ontogeny. The highly specific pattern of expression of each RXR isoform suggests a particular role for them in BAT ontogeny.

Key words: Retinoic acid receptors. T_3R. RXR. Brown adipose tissue. Uncoupling protein. Development fetal.

RESUMEN

La T_3 es un activador transcripcional del gen de la proteína desacopladora (*ucp*) de tejido adiposo marrón (TAM). Los receptores de T_3 (T_3R) y los receptores de retinoide X (RXR) interaccionan como heterodimeros con los elementos de respuesta a T_3 (TRE) en los genes diana. Los mRNA de RXRα, RXRβ y RXRγ son detectables en TAM durante el desarrollo en la rata. Los niveles de mRNA de RXRα son altos en TAM de fetos a día 18 y decrecen posteriormente. La expresión de mRNA de RXRβ no varia con el desarrollo. Los niveles de mRNA de RXRγ presentan un marcado aumento entre los días 18 y 20 de vida fetal, coincidiendo con el inicio de la transcripción del gen *ucp*. Paralelamente, los niveles de T_3R α y β_1 también aumentan entre día 18 y 20. Estos resultados sugieren que los RXRs pueden estar involucrados en la regulación de la transcripción del gen *ucp* por T_3 durante la ontogenia. La alta especi-

ficidad de los perfiles de expresión de cada isoforma RXR es compatible con una función particular para cada uno de ellos en la ontogenia del TAM.
Palabras clave: Receptores de ácido retinoico. T_3R. RXR. Tejido adiposo marrón. Proteína desacopladora. Desarrollo fetal.

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INTRODUCTION

Brown adipose tissue is the main site for non-shivering thermogenesis in mammals. The thermogenic capacity of brown fat is due to the presence of the "uncoupling protein" (UCP), a mitochondrial protein that uncouples oxidative phosphorylation from the respiratory chain, causing energy dissipation as heat (1). This source of heat production is especially important during the neonatal period, when the newborn is exposed to the thermal conditions of extrauterine life (2).

The thermogenic activity of brown adipose tissue is regulated mainly by sympathetic stimulation, although it is also controlled by other factors (1). The onset of UCP gene transcription, which may be considered as the key point of brown fat differentiation *in vivo*, begins between days 18 and 19 of fetal life in the rat and is followed by an abrupt increase in the UCP mRNA levels at day 20 (3). Considering that the sympathetic nervous system innervating brown adipose tissue is not fully developed in fetal life (2), the modulation of UCP gene expression in this period must have particular features with respect to the predominance of the adrenergic regulation in adult animals. In this key moment of brown fat differentiation there is a dramatic increase in the expression of several transcription factors, such as CCAAT/Enhancer binding proteins (C/EBP) -α and -β and T_3 receptors (T_3Rs) (4, 5), that have been reported to influence the *ucp* gene promoter activity (6-8). In fact, the profiles of iodothyronine 5'-deiodinase, responsible of the local conversion of T_4 to T_3, nuclear T_3 content and T_3 receptor occupancy also show peak values in fetal BAT at day 20 (5). Thus, T_3 signalling pathway may be important for the ontogenic development of brown fat. Recently, Rabelo *et al.* (8) have identified two thyroid response elements (TREs) located in the distal enhancer of the rat *ucp* gene. The T_3 stimulatory effect on the *ucp* gene is mediated by T_3R-Retinoid X receptor (RXR) heterodimers acting on both TREs (8). Thus, expression of RXRs can influence T_3 action.

RXRs belong to the superfamily of nuclear receptors. RXRs can act as homodimers or as heterodimeric partners of other members of the nuclear receptor superfamily, such as T_3Rs or retinoic acid receptors (RARs), and

may be key actors involved in the mediation of several hormonal signals (9). RXRs are encoded by three different genes (α, β and γ). T_3Rs are encoded by two separate genes c-*erb*Aα and -β. Distinct mRNA species can be generated by differential processing of transcripts and/or initiation of translation, resulting in multiple forms of RXRs and T_3Rs, which may differ in their tissue and developmental specific pattern of expression (10-12). In the present report, we determine the pattern of RXRs and T_3Rs mRNA expression during fetal and postnatal development of rat brown adipose tissue.

MATERIALS AND METHODS

Animals

Female rats (180-200g each) were mated and the day of pregnancy was determined by the presence of spermatozoa 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, 21 °C). When the prenatal period was studied, fetuses were obtained by Caesarian section on days 18 and 20 of gestation. Fetuses were killed by decapitation, and the interscapular brown adipose tissue was removed and immediately frozen in liquid nitrogen. When the postnatal period was studied, pups remained with their mother after spontaneous delivery and were sacrificed 15 days after birth. Adult male rats (200g) were used as controls.

RNA isolation and Northern Analysis

Total RNA was prepared from frozen tissue by homogenization in 4M guanidine thiocyanate (13). RNA samples (25 ìg per lane), containing 0.2 ìg/ml ethidium bromide for easy monitoring of the RNA (14), were subjected to electrophoresis on 1.5% agarose-formaldehyde gels and transferred to nylon membranes (N^+, Boehringer-Mannheim). Filters were prehybridized and hybridized in 0.25 M Na_2HPO_4 (pH 7.2), 1mM EDTA, 20% SDS, 0.5% blocking reagent (Boehringer-Mannheim)(15). Filters were washed under stringent conditions (20 mM Na_2HPO_4 (pH 7.2), 1mM EDTA, 1% SDS, 55 °C, 30 min.) and then exposed to autoradiography. After stripping, the blots were successively rehybridized. The following full-length cDNA probes were used: mouse RXRα, RXRβ, RXRγ (16), rat c-erb Aα and -β (11). cDNA probes were labelled with [α-^{32}P]dCTP using the random oligonucleotide-primer method. All probes were used for hybridization at 2 x 10^6 c.p.m./ml. Autoradiographs were quantified by densitometry (Phoretics). A minimum of three independent detections were performed.

RESULTS

Hybridization with the RXRα probe resulted in the detection of a single mRNA species of ~5.6 kb. The RXRα mRNA expression was high in BAT from 18-day-old fetuses and decreased sharply at day 20, when values were lower than those in 15-day-old pups and adult rats (Figure 1.A). Two RXRβ mRNA species of ~3.0 and ~2.7 kb were detected in rat BAT. RXRβ mRNA levels were similar in BAT at any stage of development (Figure 1.B). Two mRNA species of ~2.5 and ~2.0 kb have been described for RXRγ (10), and both mRNA isoforms were detectable in BAT from 18-day-old fetuses, but only the 2.0 kb species was detectable in BAT from the other stages of development studied. RXRγ mRNA levels in 18-day-old fetuses were low, and increased between days 18 and 20 (Figure 1.C). RXRγ mRNA levels at day 20 were similar to those in 15-day-old pups. In adult rats, RXRγ mRNA abundance was similar to that in 18-day-old fetuses.

Subsequent hybridization of rat BAT RNA with the c-*erb*Aα probe resulted in detection of the ~6.6 kb α, ~5.5 α_1 and ~2.6 kb α_2 mRNA species previously described (5). The levels of the three c-*erb*Aα mRNA species were similar in BAT from 18-day-old fetuses and adults (Figure 2.A-C). Maximal values were observed on day 20. In 15-day-old pups the concentrations of these mRNA species were higher than those in adults. Hybridization with the c-*erb*A ß probe resulted in the detection of a single mRNA species of ~6.5 kb ($ß_1$), as previously described (5). $ß_1$ mRNA was very faintly expressed in samples from 18-day-old fetuses and there was a sharp increase between days 18 and 20 (Figure 2.D). The levels on day 20 were higher than those in adult rats.

DISCUSSION

The aim of the present paper was to determine the ontogenic profiles of RXRs mRNA expression in rat BAT. The study was focused on the three types of RXR due to its potential role as partners of T_3Rs in the transcriptional regulation of the *ucp* gene as well as other T_3 target genes (8,9). Our results show that RXRs mRNAs are already expressed in rat BAT during fetal development. The ontogenic profiles of RXRs mRNA expression show specific features for each RXR receptor type. RXRα mRNA levels are higher in 18-day-old fetuses than in any other stage, and decreased sharply at day 20. In contrast, RXRγ mRNA levels increase between days 18 and 20 of fetal life, concurrently with the increase in T_3Rs expression in BAT. Finally, RXRβ mRNA levels do not vary significatively during BAT development. Previous studies have shown that RXRα and RXRβ mRNAs are widely expressed in adult rodents, whereas *RXRγ presents a more restrictive pattern of expression,*

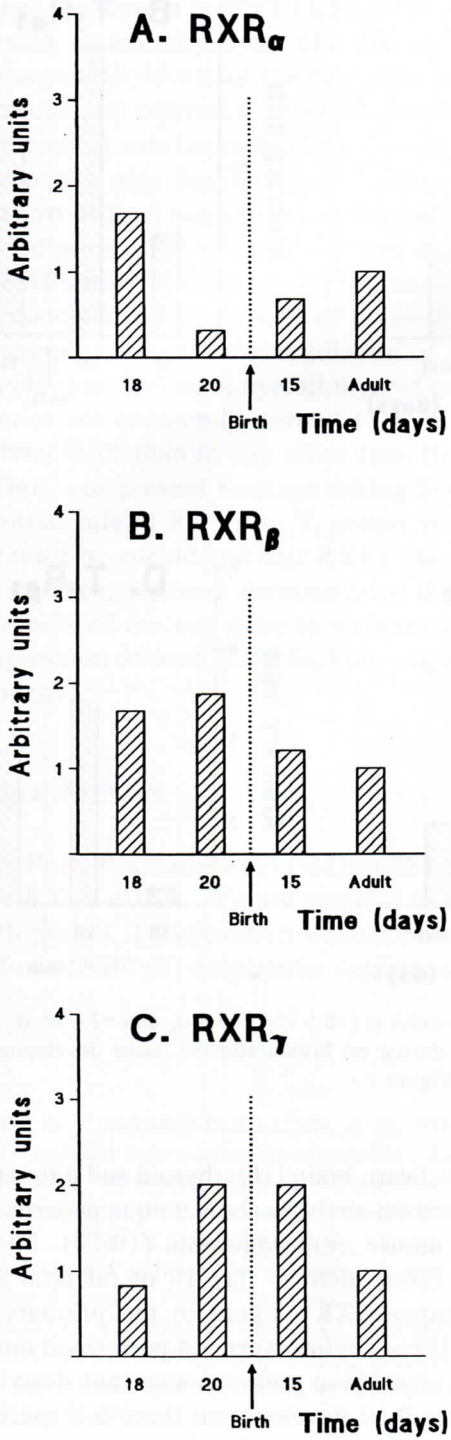

Fig. 1.—Quantitative changes in RXRα, RXRβ and RXRγ mRNA expression during rat brown adipose tissue development. Results are expressed relative to the mean value in brown adipose tissue of adult rats, setting as 1.0 for each RXR mRNA. For each RNA preparation the interscapular brown adipose tissues of the entire litters were pooled (for fetal groups at 18 and 20 days of gestation), and three animals were pooled for the postnatal points.

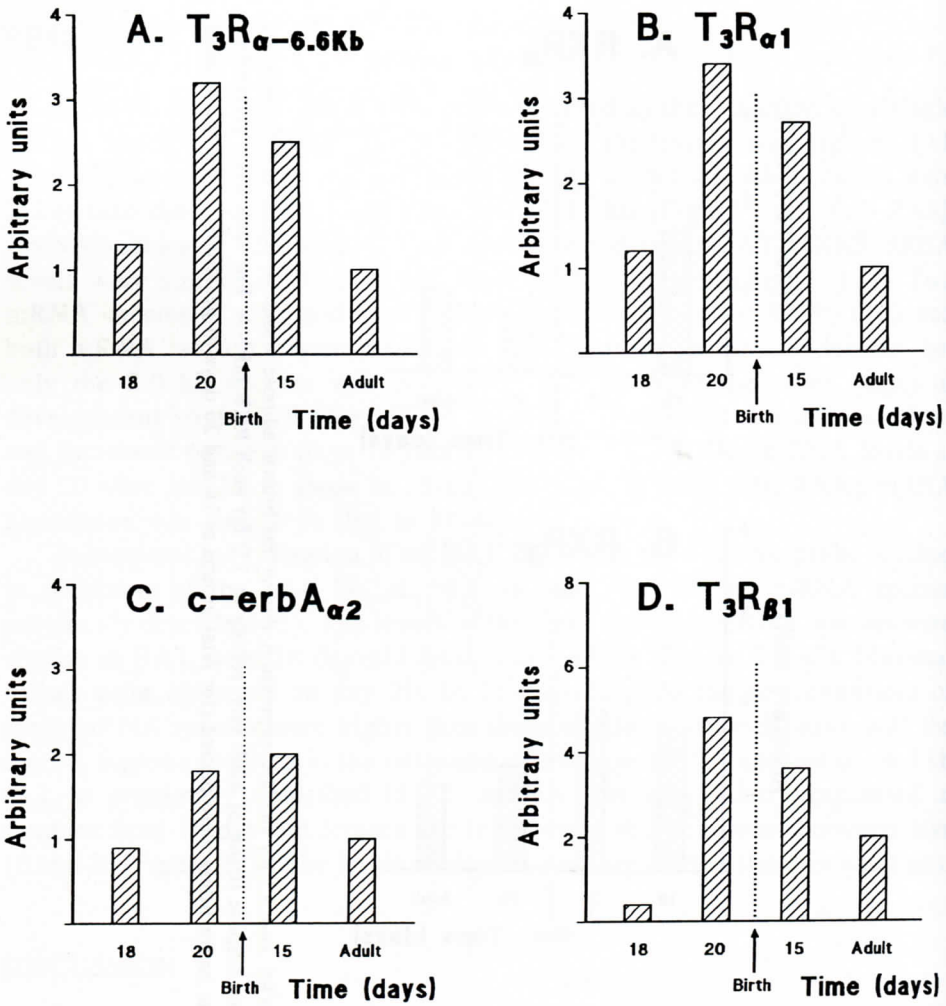

Fig. 2.—Quantitative changes in c-*erb*A α (~6.6 kbα, ~5.5 α_1 and ~2.6 kb α_2 mRNA species) and c-*erb*A β^1 mRNA expression during rat brown adipose tissue development. For experimental details, see the legend to Figure 1.

being localized only in muscle, heart, brain (10), thyroid and pituitary glands (17) and BAT (18). In situ hybridization analyses show unique patterns of expression for each RXR type during mouse embryogenesis (10,19). Interestingly, in 16.5-day old mouse fetuses (equivalent to day 19 of rat fetal development) autoradiographs shown a strong RXRγ signal in the pituitary and thyroid glands (10,19). However, as these studies were not performed during late fetal stages, BAT developmental expression patterns were not described.

Between days 18 and 20 of rat fetal development there is a specific induction

of the *ucp* gene expression in BAT (3,5). UCP is considered the main marker of differentiated brown adipocytes (1). During the same period, there is an increase in the mRNA levels of the two T_3R: α (6.6 kb and α_1 species) and $ß_1$ (present results and reference 5) which correlate with the increase in T_3Rs levels, determined as maximum binding capacity (5). Nuclear T_3 content and receptor occupancy also reached peak values at day 20 (5). Indeed, this ontogenic pattern of fetal acquisition of the euthyroidal tissue status is unique when compared with other thyroid sensitive tissues (5,20).

Among RXRs mRNA expression profiles, only RXRγ mRNA expression pattern is in concordance with those of α and β T_3Rs. It has been suggested that RXRγ may play a role in the regulation of thyroid hormone target genes in thyrotropes (17) as well as in overall thyroid hormone homeostasis, although RXRγ null mice are apparently normal (21). In fact, tissue T_3 concentration is higher in fetal BAT than in any other fetal tissue, except the thyroid gland itself (22). Thus, our present findings during BAT development are compatible with a similar role of RXRγ on T_3 action and/or production in fetal BAT. Moreover, it must be considered that RXRs can participate in other hormonal signals. For instance, we have demonstrated that RXRs are also involved in the responsiveness of the *ucp* gene to retinoic acid (23). The highly specific pattern of expression of each RXR isoform suggest a particular role for them in BAT ontogeny.

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