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Peroxisome proliferator-activated receptor-α expression in rat liver during postnatal development

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Abstract — The expression of the peroxisome proliferator-activated receptor-α (PPARα) as well as of some related genes was studied in rat liver at different stages of development (from 19-day-old fetuses to 1 month-old rats). The level of PPARα mRNA appeared higher in neonates than in fetuses or 1 month-old rats. Whereas the pattern for phosphoenolpyruvate carboxykinase (PEPCK) mRNA level was similar to that of PPARα, the mRNA level of both acyl-CoA oxidase (ACO) and apolipoprotein CIII (apo CIII) showed diverse profiles. Western blotting analysis also revealed an increased level of PPARα protein in liver of suckling rats. Similarities of mRNA PEPCK and PPARα expression indicate a common control mechanism, where both nutritional and hormonal factors may be involved. © 2000 Société française de biochimie et biologie moléculaire / Éditions scientifiques et médicales Elsevier SAS

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1. Introduction

Peroxisome proliferator-activated receptors (PPAR) are members of the steroid nuclear receptor superfamily, which is a large class of ligand-activated transcription factors regulating gene expression. Both peroxisome proliferator compounds and fatty acids, or their derivatives, are known to activate these receptors either indirectly or as ligands [1], therefore acting on gene regulation in a similar way to steroid hormones. Induced PPARs regulate the expression of genes encoding proteins related to lipid metabolism. So far, three PPAR subtypes have been identified in the rat: PPARα, β and γ. The isoform α is the best-characterized and it is primarily expressed in tissues like liver having a high fatty acid catabolism activity, being involved in the modulation of that pathway. Moreover, it has been postulated that PPARs might be responsible for the cellular fuel selection in the context of the substrate competition between fatty acids and glucose [2].

The hormonal regulation of PPARα expression has been poorly described to date, and only glucocorticoids seem to show a clear effect [3]. Furthermore, most studies have been carried out in vitro and controversial results have been reported regarding the regulation of the expression of PPARα by either insulin [4–6] or by fatty acids [5, 7]. Physiological conditions of modified plasma insulin and fatty acid levels are therefore desirable in order to test whether these factors control PPARα expression in vivo.

During perinatal development, both the transition from fetal to neonatal stage, and of suckling period to weaning are known to modify endocrine and nutritional conditions that could influence the pattern of expression of PPARα as it has been described for other genes related to PPARα, like phosphoenolpyruvate carboxykinase (PEPCK) [8]. A previous work studied the pattern expression of PPARα in rats during embryonic development [9], and it showed an increasing expression related to aging. Another report, using in situ hybridization analysis [10], showed an increasing expression of PPARα in mouse liver from the fetal stage to adulthood. By measuring the specific mRNA and protein levels, the present study was addressed to determine the expression of PPARα in rat liver throughout the perinatal and suckling transitional stages. The study was extended to determine the mRNA level of genes whose expressions have previously been proposed to be related to PPARα: acyl-CoA oxidase (ACO), apolipoprotein CIII (apo CIII), and PEPCK [11].

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats weighing 160–190 g were mated. Mothers and their fetuses were killed by decapitation at different post-fecundation times: 19, 20 and 21 days. On the day of birth, each litter was reduced to nine pups per mother and pups were killed at 0, 1, 2, 5, 10, 15, 20, 25, and 30 days after birth. On the 20th day of life,

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the lactating mothers were removed from the cages. In all cases, the liver was immediately removed, placed into liquid nitrogen and stored at –70 °C until analysis.

The experimental protocol was approved by the Animal Research Committee of the Faculty of Experimental Sciences, University of San Pablo-CEU.

2.2. *RNA analysis*

Total cellular RNA was prepared either from the liver of individual animals (rats of 5 to 30 days of age) or from liver pools of three animals of the same age (fetuses and newborns from day 0 to 2 days of age). Total RNA was isolated by a modification of the guanidium isothiocyanate method using Ultraspec RNA according to the manufacturer's instructions (Biotecx Labs, Houston, USA). Around 25 μg of total RNA was denatured, separated on 1.2% agarose gels and transferred to nylon membranes in 20 × SSC. The cDNA probes for Northern blot analysis were labeled with [α-^{32}P]dCTP by using the random primer DNA labeling kit provided by Amersham. Probes used were: a fragment of rat ACO cDNA (kindly supplied by H.N. Sorensen, University of Oslo, and with permission of T. Osumi, Humeji Institute of Technology, Hyogo, Japan), rat apo CIII cDNA fragment (a generous gift of Dr. J. Auwerx, Institute Pasteur, Lille, France), rat PEPCK cDNA fragment (kindly supplied by Dr. D.K. Granner, Vanderbilt University Medical Center, Nashville, USA), and a fragment of rat PPARα cDNA (a generous gift of Dr. J-Å Gustafsson, Karolinska Institute, Huddinge, Sweden). Blots were prehybridized at 42 °C for 2 h and hybridized overnight at the same temperature. Washing conditions were 2 × SSC, 0.1% SDS at 42 °C for 15 min twice for PPARα, and 0.1 × SSC, 0.1% SDS at 53 °C for 15 min three times for the other probes, and membranes were exposed to Kodak X-OMAT films at –70 °C from 1 h to 3 days. Filters were stripped of label at 75–80 °C for 1 h with 0.1 × SSC, 0.5% SDS, 0.1% tetrasodium pyrophosphate and then rehybridized.

2.3. *Immunoblotting*

Liver samples were homogenized in a buffer containing 25 mM MOPS, 1 mM EDTA, 2mM DTT, 0.4 M KCl, pH 7.9, and 0.2 mM PMSF. Sixty μg of total protein of liver homogenate were denatured and subjected to SDS-PAGE. Proteins were transferred to nitrocellulose and visualized by Ponceau Red to confirm transfer. Blots were incubated with a polyclonal antibody to PPARα (Santa Cruz Biotechnology, Inc., USA), followed by a peroxidase-conjugated rabbit anti-goat antibody (Sigma) and the immunoreactive bands were visualized by enhanced chemiluminescence (ECL, Amersham).

3. Results and discussion

Experiments carried out in our laboratory showed that the amount of specific messenger RNA for PPARα was higher in liver of rat fetuses than in their mothers or adult female rats (Panadero M.I., Bocos C. and Herrera E., unpublished results). These findings were in contrast to those of Beck et al. [10], showing a progressively increasing expression of this nuclear transcriptional factor in rodents from embryonic to post-natal and adult stages. In order to clarify this controversy, the expression of PPARα and some of its target genes were studied by Northern blot analysis in liver from fetuses, newborns, suckling pups and adult rats. As shown in *figure 1*, the already appreciable level of PPARα mRNA in fetuses rapidly increased at birth, reaching the highest value around day 1 and remaining high during the first 10 days of life, to decline at day 20, remaining low at day 30 of life.

The expression of few PPARα target genes is also summarized in *figure 1*. The pattern of PEPCK mRNA throughout development paralleled that of PPARα mRNA, being practically absent during fetal life and rapidly increasing at birth and remaining high until day 10 of life, to decline thereafter. This pattern in PEPCK mRNA resembles the profile already found by others [8], reflecting the lack of gluconeogenesis during the fetal period and its rapid increase after birth, remaining enhanced during suckling and declining at weaning. This pattern may be related to the proposed influence of low insulin and high fatty acid levels in the gene expression of PEPCK during suckling [12, 13], also supporting the role of these hormonal and metabolic factors controlling the PPARα expression.

As also shown in *figure 1*, the profile of ACO mRNA throughout development was slightly different than either PPARα or PEPCK, since its highest increase occurs at day 0 and there is a progressive decline thereafter. This picture is similar to the one previously reported by Staels et al. [14]. The early peak in the mRNA level of ACO could be related to an enhanced hepatic peroxisomal fatty acid catabolism during suckling, providing an additional mechanism by which the pups oxidize milk fatty acids. An increase in the mRNA expression of 17β-hydroxysteroid dehydrogenase type IV, an enzyme that has also been implicated in the peroxisomal fatty acid β-oxidation [15] and whose expression has already been demonstrated to be regulated by PPARα in liver [16], was also observed in dot blot analysis (data not shown), although later than the peak seen in the expression of ACO. In fact, an enhanced peroxisomal capacity for fatty acid β-oxidation during suckling has already been found in both piglets and rats [17].

As also shown in *figure 1*, the pattern of apo C III mRNA expression appeared quite different from that of PPARα, being practically absent in the fetus and progressively increasing after birth to attain the highest level at 20 days of age, to values that do not differ from those in 30-day-old rats. This pattern coincides with a similar one previously reported and agrees with the known fact that

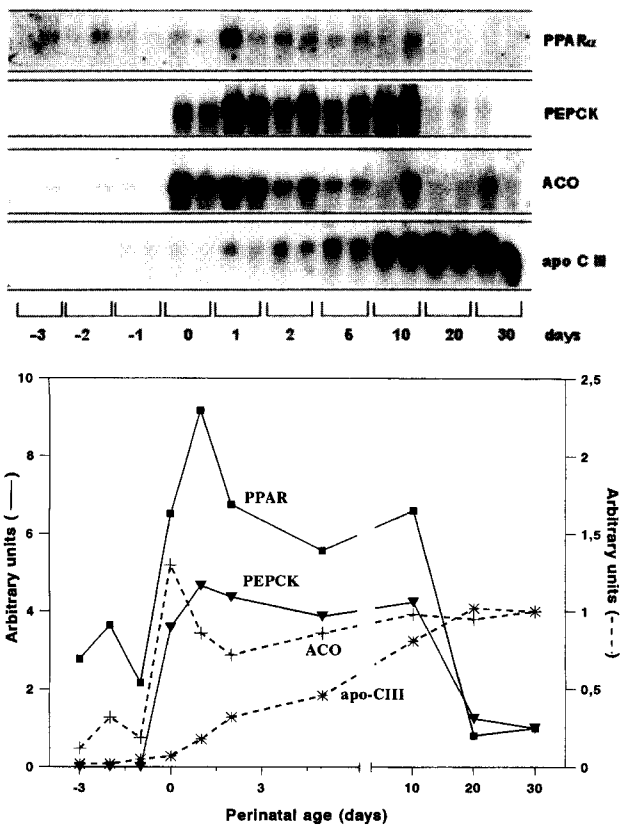

Figure 1. A. Northern blot experiments for mRNAs of PPARα, ACO, apo CIII, and PEPCK. The autoradiograms shown represent the results obtained for four animals per group. Total RNA isolated from liver of fetuses, pups and adult rats were processed as described in 2. *Materials and methods.* The developmental time-points considered and probes used are indicated. **B.** Relative amount of corresponding mRNAs obtained by densitometric scanning of the blots and normalized versus 28S ribosomal RNA ethidium bromide staining. Values are represented in arbitrary units, since the value corresponding to the 30-day-old rats is taken as 1 unit.

apo C III is negatively regulated by compounds that activate PPARα [14].

To check whether the augmented amount of PPAR mRNA in liver during suckling was also reflected by the amount of the corresponding protein, a Western blotting analysis was carried out on the same liver samples. As shown in *figure 2*, although PPARα protein is already present in fetuses, as occurred for PPARα mRNA, it appeared more abundant in the liver of suckling rats than in fetuses and weaning rats. The maximal peak in PPARα protein appeared in the liver of the 10-day-old pups, which is later than the peak found in the mRNA level, occurring around the first day of extrauterine life *(figure 1)*. This delay in the appearance of the peak in protein as compared

Figure 2. Whole-cell extracts from the liver of fetuses, pups and adult rats were separated by SDS-PAGE, transferred to nitrocellulose and probed with antibody against PPARα, followed by ECL visualization. The developmental time-points considered are indicated. The immunoblot shown is representative of four animals per group.

to mRNA of PPARα might be due to different turnover rates or differences in the translation efficiency of mRNA, and agrees with similar discrepancies previously described [18, 19].

The profile of PPAR expression during development found here could be inversely related to the pattern of insulin concentration in plasma, which is high in fetuses, followed by a reduction after parturition, reaching the lowest level at 5–10 days of age, to progressively increase again until the 30th day of life [20]. Since there are reports showing inhibitory insulin effects on PPAR expression in vitro [4–6], our results would suggest a physiologic inhibitory effect of insulin on PPARα expression. An influence of growth hormone (GH) cannot be ruled out either, since its plasma concentration increases around the onset of rat puberty [21], which occurs in the strain of rats used here (Sprague-Dawley) around the 30th day of life, at the time when the lowest expression was found here, and in vitro experiments have previously demonstrated an inhibitory effect of GH on the expression of PPARα [22]. The profile observed here for the expression of PPARα mRNA in liver throughout development may also be related to nutritional changes. Whereas during fetal and post-suckling periods rats are mainly fed with a carbohydrate-rich diet, during suckling the diet is highly lipid-based, and therefore it may be hypothesized that the PPARα transcription during this stage is enhanced by dietary fatty acids in a similar way as has been demonstrated for the expression of a number of genes, such as PEPCK [12]. In fact, previous in vitro studies have already shown that some fatty acids are able to induce PPAR expression in hepatic cells [4, 5]. Experiments designed to elucidate between these possibilities are currently being performed in our laboratory.

Besides its hormonal regulation, the high levels of PPARα mRNA and protein found here in rat neonates would indicate the existence of an additional regulating factor such as the nutritional changes occurring during this period of life. Present results therefore indicate that PPARα mRNA expression during the perinatal period is under both hormonal and fatty acid control in an analogous manner to the regulation of some of its putative target genes.

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