BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **230,** 155–158 (1997)
ARTICLE NO. RC965906

PPARα Mediates Peroxisome Proliferator-Induced Transcriptional Repression of Nonperoxisomal Gene Expression in Mouse

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Received December 3, 1996

The strain difference, peroxisome proliferator specificity and role of PPARα in peroxisome proliferator-induced transcriptional repression of nonperoxisomal transthyretin and α_{2u}-globulin genes were examined. The genes were repressed by four peroxisome proliferators in all seven mouse strains tested. The extent of repression was strongly dependent on both the mouse strains and type of proliferator, although the mRNA levels of PPARα and its partner in heterodimerization, RXRα were not different. The role of PPARα in repression was confirmed by the finding that PPARα-null mice were not responsive to transcriptional repression. These results indicate that PPARα plays an obligatory role in transcription of various genes, some of which are not related to lipid metabolism.

All structurally diverse peroxisome proliferators (PPs) are thought to induce transcription of the peroxisomal β-oxidation enzyme genes through a similar mechanism. Cloning of the peroxisome proliferator-activated receptor (PPAR) α (1), transcriptional analysis of the genes (2,3) and development of a PPARα-null mouse (4) have revealed that the mechanism of the transcriptional activation mediated by PPARα and PP-response elements is through dimerization with retinoid X receptors (RXRs). Subsequent cloning of other types of PPARs (5,6) and the discovery of new target genes (7,8) have revealed the central roles of the PPARs in lipid metabolism and adipocyte differentiation by activating target genes depending on respective ligands (9,10).

In the rat liver, PPs not only induce transcription of the genes encoding lipid metabolizing enzymes, but also repress several other genes. We found that apoE is transiently down regulated by various proliferators (11). ApoAI, apoAIV (8) and apoCIII (12) have been reported to be down regulated by the proliferators. In addition to these proteins related to lipid metabolism, we have shown the immediate down regulation of transthyretin (13) and the endoplasmic protein, BiP/GRP78 (14), both of which are not apparently involved in lipid homeostasis. Alvares *et al.* reported that α_{2u}-globulin is down-regulated by ciprofibrate in the rat liver (15), whereas we detected little effect of clofibrate on the expression of this gene (13). They also showed that α_{2u}-globulin gene transcription was significantly repressed by the PP (15).

We have been studying non-peroxisomal cellular events mediated in the rodent liver by peroxisome proliferators, because we believe that the changes induced in fundamental and/or liver-specific cellular functions are involved in hepatomegaly and hepatocarcinogenesis (16). Atypical transcriptional modulation of known genes are not likely to be directly involved in such events. However, analyses of their mechanisms may resolve general problems. In this study, we investigated the factors that influence the extent to which the α_{2u}-globulin gene is repressed, using several mouse strains and various peroxisome proliferators. We also examined the involvement of PPARα in repression using a knockout mouse.

MATERIALS AND METHODS

Materials. Clofibrate (2-(*p*-chlorophenoxy)isobutyric acid ethyl ester), Wy14,643 (4-chloro-6-(2,3-xylidino)-2-pyrimidinyl-thio)acetic acid, DEHA (di(2-ethyhexyl)adipate), and DEHP (di(2-ethylhexyl)phthalate) were purchased from Tokyo-Kasei (Tokyo, Japan).

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Abbreviations: DEHA, di(2-ethyhexyl)adipate; DEHP, (di(2-ethylhexyl)phthalate; HD, peroxisomal hydratase-dehydrogenase; PP, peroxisome proliferator; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; TTR, transthyretin; Wy14,643, 4-chloro-6-(2,3-xylidino)-2-pyrimidinyl-thio)acetic acid.

0006-291X/97 $25.00

Animals and treatment. Seven strains of normal male mice (5-6 weeks old; BALB/c, C57BL, C3H, DBA, CBA, NZB, and NZW) were kept on a 12-h light-dark cycle with free access to water and food. The control diet was CE7 (Clea Japan) and the others were C7 containing Wy14,643 (0.1%), clofibrate (0.5%), DEHA (2%), or DEHP (2%). Animals were sacrificed at 1:30 p.m. to minimize the effect of diurnal rhythms (17). In sudies using PPARα gene knockout mice, male PPARα (−/−) mice or (+/+) (F3, Sv/129 strain homozygotes or wild-type; 10-12 weeks of age) were fed with a diet containing 0.1% Wy14,643 for 2 weeks as described previously (4).

RNA preparation and Northern blots. Total RNA prepared from the liver by the acid guanidium thiocyanate-phenol-chloroform extraction method (18) was subjected to Northern blotting essentially as described (14). Some plasmid DNAs used for probes were obtained by cloning of PCR products of cDNA synthesized from poly(A) RNA isolated from the liver of Wy14,643-fed NZW mice. Their identities were confirmed by sequencing 300-400 bases from both ends. The synthetic oligonucleotides used to amplify respective cDNA sequences were 5′)GTCAACTCCCTCAGGAGCGTCTTGG and 5′)-GGAACGTAGAAAGCCAGGGATCAG for peroxisomal hydratase-dehydrogenase (HD, corresponding to nucleotides 861 to 1816 of the published rat sequence (19); 5′)CCACTA(C/T)GGAGTTCA(C/T)-GC(A/T)TGGTG and 5′)GTAGATCTC(C/T)TG(G/C)AACAG(G/T)GG-GTG for PPARα (corresponding to nucleotides 502 to 1559 (1)); 5′)TGGGTCC(G/C)CCCTTCTC(A/T)GTCATCAG and 5′)GCCTC-(A/C)AGCATCTCCATGAGGAAGG for RXRα (corresponding to nucleotides 325 to 1480 (20)). Other plasmids were as described (13, 14). After hybridization, the membranes were washed and autoradiographed with an intensifying screen at −80°C. Bands were quantified using a BAS2000 image analyzer (Fujix, Japan).

RESULTS AND DISCUSSION

Strain Differences in PP-Induced Downregulation of α_{2u}-Globulin

Repression of α_{2u}-globulin gene transcription by ciprofibrate in the rat liver was reported by Alvares *et al.* (15), whereas we detected little effect of clofibrate on gene expression. To examine the causes of this apparent discrepancy, we first confirmed that an another type of PP, Wy14,643, moderately repressed α_{2u}-globulin gene transcription in the mouse strain C57BL (not shown). We then compared the responsiveness of 7 mouse strains to Wy14,643. The changes in the levels of several mRNAs, including transthyretin (TTR) and α_{2u}-globulin, are summarized in Fig. 1. Variations among individuals (n=3) were small (usually less than 15% of the largest value) and representative profiles obtained from one RNA sample from each group are shown. All strains responded well to Wy14,643 as determined by the level of induction of mRNAs encoding the peroxisomal β-oxidation enzyme, enoyl-coenzyme A hydratase-3-hydroxyacyl-coenzyme A dehydrogenase (HD). The mRNA level was increased nearly 100-fold in the liver of BALB/c mice within 5 days (highest) and 75-fold in NZW mice (lowest). During this period, the levels of apoE mRNA did not change significantly and served as internal control in this study.

The levels of TTR mRNA in most strains were downregulated by Wy14,643 as they were in rat when clofibrate was administered (13). Although control levels of

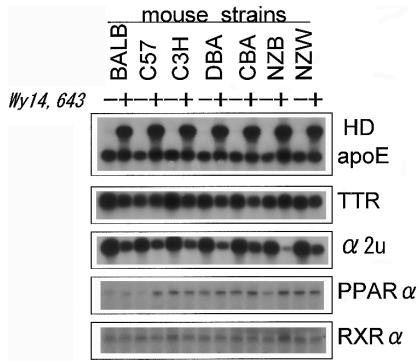

FIG. 1. Strain differences in responsiveness to a peroxisome proliferator, Wy14,643. Seven mouse strains (BALB/c, C57BL, C3H, DBA, CBA, NZB, and NZW) were fed with a control diet (−) or a diet containing Wy14,643 (+) for 5 days. Total RNA (5 μg) from individual livers was Northern blotted using the cDNAs for peroxisomal HD (HD), apoE protein (apoE), transthretin (TTR), α_{2u}-globulin (α_{2u}), PPARα, and RXRα.

TTR mRNA were similar among the 7 strains, the extent of downregulation differed: There was a nearly 40% decrease in the liver of the CBA mouse (highest) and little decrease in the C57 mouse. There was no correlation between the extent of induction of HD mRNA and that of TTR mRNA reduction.

The Wy14,643-induced changes in the levels of α_{2u}-globulin mRNA showed a large strain difference though the control levels were similar. In the NZB mouse liver, the mRNA level was reduced to 5% of the control level within 5 days, whereas it was to 30-50% in the livers of other mice. The strain difference in this response, however, was not due to the levels of two transcription factors, PPARα and RXRα, which bind to PP-response element on the target genes (3), because the levels of mRNAs for these proteins were not significantly different among strains, or between control and Wy14,643-treated animals. TTR and α_{2u}-globulin were not repressed in the same manner, suggesting the involvement of distinct factors in each.

Time Course of Repression

To compare the time courses of induction of HD mRNA and repression of α_{2u}-globulin mRNA, the most sensitive NZB mice were fed Wy14,643 for various periods and the levels of these mRNAs were measured by Northern blotting (Fig. 2). The level of α_{2u}-globulin mRNA decreased to less than 1% of control level in 10 days and this level was maintained throughout the course of feeding Wy14,643. In contrast to a rapid increase of HD mRNA level reaching the maximal level in one day, the α_{2u}-globulin mRNA was reduced to half of the control over two days and it became undetectable level by 10 days. The half-life of α_{2u}-globulin mRNA is not known, but transcription of α_{2u}-globulin gene was not likely to be completely shut off immediately by

Wy14,643. Differences in these time courses can be explained either by immediate reduction of the transcription rate to cause a gradual decrease of the mRNA or by secondary inhibition of the transcription after a time lag to cause the delayed decrease.

After withdrawal of a peroxisome proliferator, the α_{2u}-globulin mRNA started reappearing after a long time lag of several days as reported by Alvares *et al.* (15). The recovery process seems to be too complicated to study the underlying mechanism of the repression.

Peroxisome Proliferator Specificity

The extent by which the α_{2u}-globulin mRNA levels was reduced also differed among the PPs. The effects of various kinds of PPs on the mRNA levels are shown in Fig. 2. All PPs reduced the level, but Wy14,643 and DEHP were most effective, followed by clofibrate and DEHA.

Thus transcription of the α_{2u}-globulin gene was repressed in all mouse strains tested by various PPs, but the extent was largely strain and PP-type dependent. These are probably responsible for the apparent discrepancy between our results (13) and those of Alvares *et al.* (15).

Influence of PPARα Gene Knockout on PP-Induced Repression

We examined the involvement of PPARα in transcriptional repression using a PPAR-null mouse (4). Northern blots of liver RNA from wild type (+/+) and PPARα-null mice (−/−) fed with a control diet or that containing Wy14,643, are shown in Fig. 3. HD mRNA increased only in wild type mice (+/+) fed Wy14,643 but not in the null animals as previously reported (4). TTR mRNA slightly decreased (about 25% of control) and α_{2u}-globulin mRNA almost disappeared (less than 5%) in (+/+) mice given Wy14,643 as in Fig. 2, whereas Wy14,643 did not induce decrease in (−/−) mice. These results establish that PPARα is involved in PP-induced repression of TTR and α_{2u}-globulin gene transcription in the mouse liver.

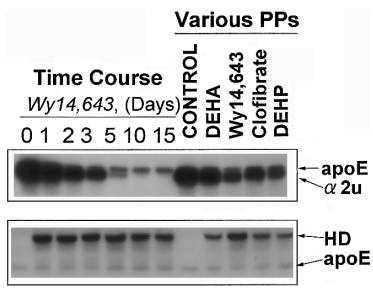

FIG. 2. Time course of α_{2u}-globulin mRNA repression by Wy14,643 and effects of various PPs. NZB mice were fed with a diet containing Wy14,643 for 1-15 days as indicated, or containing the indicated PP for 5 days. Total RNA isolated from individual livers was Northern blotted using the 4 cDNA probes as described in the legend to Fig. 1.

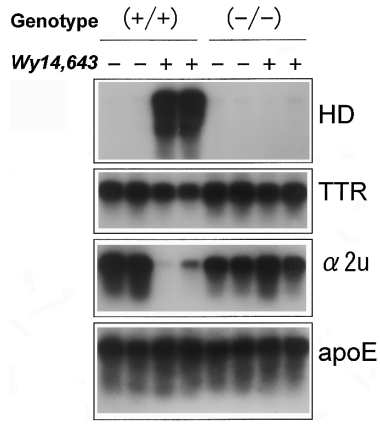

FIG. 3. Influence of PPARα gene knockout on PP-induced transcriptional repression. Wild type (+/+) and PPARα gene knockout (−/−) mice were fed with a control diet (−) or that containing Wy14,643 (+). Total RNA from individual livers was Northern blotted using the 4 cDNA probes as described in the legend to Fig. 1.

Involvement of PPARα in transcriptional repression can be direct or indirect. In an indirect mechanism, for example, PPARα may be involved in the transcriptional repression of the genes encoding factors necessary for TTR and α_{2u}-globulin gene transcription. Thus transcription of these genes is secondarily repressed by PPs. In a direct mechanism, PPARα may bind to the regulatory regions of these genes, inhibiting transcription with or without RXRα but with respective unidentified factors, of which the levels differ among mouse strains. Alternatively, the transcriptional machineries for the TTR and α_{2u}-globulin genes may share an integrating transcription factor, such as a cointegrator (21), with those for peroxisomal β-oxidation enzyme genes and the amount of the factor is the limiting step in the transcriptional activities.

Further studies, including quantitative comparison of the PP-induced changes in transcriptional rates in the activated HD and the repressed α_{2u}-globulin gene expression and functional analysis of the regulatory region of TTR and α_{2u}-globulin genes, are necessary to elucidate the mechanism of PP-induced transcriptional repression. We believe that these studies are important for understanding the diverse effects of PPs such as hepatomegaly and hepatocarcinogenesis.

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