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PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene

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Increased activity of lipoprotein lipase (LPL) may explain the hypotriglyceridemic effects of fibrates, thiazolindinediones and fatty acids, which are known activators (and/or ligands) of the various peroxisome proliferator-activated receptors (PPARs). Treatment with compounds which activate preferentially PPARα, such as fenofibrate, induced LPL expression exclusively in rat liver. In contrast, the antidiabetic thiazolidinedione BRL 49653, a high affinity ligand for PPARγ, had no effect on liver, but induced LPL expression in rat adipose tissue. In the hepatocyte cell line AML-12, fenofibric acid, but not BRL 49653, induced LPL mRNA, whereas in 3T3-L1 preadipocytes, the PPARγ ligand induced LPL mRNA levels much quicker and to a higher extent than fenofibric acid. In both the *in vivo* and *in vitro* studies, inducibility by either PPARα or γ activators, correlated with the tissue distribution of the respective PPARs: an adipocyte-restricted expression of PPARγ, whereas PPARα was expressed predominately in liver. A sequence element was identified in the human LPL promoter that mediates the functional responsiveness to fibrates and thiazolinediones. Methylation interference and gel retardation assays demonstrated that a PPARα or γ and the 9-*cis* retinoic acid receptor (RXR) heterodimers bind to this sequence –169 TGCCCTTTCCCCC –157. These data provide evidence that transcriptional activation of the LPL gene by fibrates and thiazolidinediones is mediated by PPAR–RXR heterodimers and contributes significantly to their hypotriglyceridemic effects *in vivo*. Whereas thiazolidinediones predominantly affect adipocyte LPL production through activation of PPARγ, fibrates exert their effects mainly in the liver via activation of PPARα.

Keywords: atherosclerosis/gene expression/lipoproteins/nuclear receptors/peroxisomes

Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the superfamily of nuclear hormone receptors that function as ligand-dependent transcription factors. Upon ligand activation they regulate the expression of genes containing specific response elements, called PPREs (Osumi *et al.*, 1991; Tugwood *et al.*, 1992). Three receptor subtypes of PPARs, termed α, δ (or β) and γ, have been identified (Isseman and Green, 1990; Dreyer *et al.*, 1992; Gottlicher *et al.*, 1992; Schmidt *et al.*, 1992; Sher *et al.*, 1993; Zhu *et al.*, 1993; Kliewer *et al.*, 1994; Tontonoz *et al.*, 1994a; Amri *et al.*, 1995; Aperlo *et al.*, 1995). These receptors heterodimerize with the retinoid X receptor (RXR) and alter the transcription of target genes after binding to PPREs, which consist of a hexameric nucleotide direct repeat of the recognition motif (TGACCT) spaced by one nucleotide (DR-1). After activation, PPARs control the expression of genes implicated in intra- and extracellular lipid metabolism, such as the genes encoding enzymes involved in the peroxisomal β-oxidation pathway (Osumi *et al.*, 1991; Tugwood *et al.*, 1992; Zhang *et al.*, 1992; Bardot *et al.*, 1993; Marcus *et al.*, 1993; Alvarez *et al.*, 1994), cytochrome P450 4A6 (Muerhoff *et al.*, 1992; Palmer *et al.*, 1994), 3-hydroxy-3-methylglutaryl-CoA synthase (Rodriguez *et al.*, 1994), medium chain acyl-CoA dehydrogenase (Gulick *et al.*, 1994), adipocyte fatty acid binding protein aP2 (Tontonoz *et al.*, 1994a), apolipoproteins A-I and A-II (Vu-Dac *et al.*, 1994, 1995) and the acyl-CoA synthetase gene (Schoonjans *et al.*, 1995).

The transcriptional activity of the PPAR subtypes is enhanced by a multitude of chemical compounds, including fatty acids, thiazolidinedione antidiabetic agents, prostaglandins, peroxisome proliferators and fibrate hypolipidemic drugs. Whereas all these compounds are known to activate PPARs, only for PPARγ has a ligand been identified. PPARγ directly binds antidiabetic thiazolidinediones (Forman *et al.*, 1995; Lehmann *et al.*, 1995) and prostaglandin derivatives (Forman *et al.*, 1995; Kliewer *et al.*, 1995), but not the other activators. Fatty acids more selectively activate the PPARδ type, whereas fibrates are more selective as PPARα activators (Kliewer *et al.*, 1994). Furthermore, the fibrate Wy-14.643 was shown to have almost no activity on PPARγ (Yu *et al.*, 1995). In addition to ligand selectivity for the PPAR subtypes, the expression patterns of the various PPAR subtypes are also distinct. PPARα is predominantly expressed in liver, heart, kidney, intestinal mucosa and brown adipose tissue, all tissues with high catabolic rates of fatty acids and peroxisomal metabolism (Isseman and Green, 1990; Beck *et al.*, 1992; Braissant *et al.*, 1995). PPARδ is abundantly and ubiquitously expressed, whereas PPARγ presents a much more restricted expression (Braissant *et al.*, 1995). In fact, the mPPARγ2 isoform is predominantly expressed in adipose tissue (Zhu *et al.*, 1993; Tontonoz *et al.*, 1994a,b). The pivotal role of mPPARγ2 in adipocyte differentiation was elegantly demonstrated by the induction of an adipocyte phenotype in fibroblast and muscle cells overexpressing

mPPARγ2 (Tontonoz *et al.*, 1994a). Furthermore, several key adipocyte-specific genes are induced by PPAR activators and contain functional PPREs in their regulatory sequences (Schoonjans *et al.*, 1993b, 1995; Tontonoz *et al.*, 1994b, 1995). Finally, arachidonic acid-derived PPARγ ligands are potent inducers of adipocyte differentiation (Gaillard *et al.*, 1989; Forman *et al.*, 1995; Kliewer *et al.*, 1995).

Fibrates, fish oils and thiazolidinediones are known to lower serum triglyceride levels (Grundy and Denke, 1990; Ikeda *et al.*, 1990; Schonfeld, 1994), but the exact mechanism by which they affect triglyceride levels is currently unknown. Whereas clinical studies suggested that part of the beneficial effects of fibrates and fish oils on lipoprotein metabolism is due to the induction of lipoprotein lipase (LPL; EC 3.1.1.34) activity (Heller and Harvengt, 1983; Sommariva *et al.*, 1983; Gnasso *et al.*, 1986; Weisweiler, 1989; Levy *et al.*, 1993; Schonfeld, 1994; reviewed in Grundy and Denke, 1990; Schonfeld, 1994), it is not known whether thiazolidinediones have a similar effect. LPL catalyzes the hydrolysis of triglycerides in lipoprotein particles into fatty acids and monoacylglycerol (reviewed in Auwerx *et al.*, 1992). Hence, the action of LPL is crucial both in energy metabolism (supply of free fatty acids) and in lipoprotein metabolism (conversion of triglyceride-rich lipoproteins). Tissue-specific expression of the LPL gene is complex and not fully understood at present. Typically, human LPL is absent in undifferentiated tissues and cells and its expression is induced upon differentiation in adipose tissue, muscle, macrophages and the mammary gland. In liver, LPL is only expressed before weaning and extinguished during liver differentiation and development (Staels and Auwerx, 1992).

Given this pivotal role of LPL in lipid and energy metabolism, we investigated the effects of two distinct chemical classes of PPAR activators, i.e. fibrates (PPARα-specific) and the antidiabetic thiazolidinediones (PPARγ-specific), on tissue-specific LPL gene expression in the rat. Fibrate treatment induced LPL expression exclusively in liver, whereas BRL 49653, the high affinity ligand for PPARγ, had no effect on liver, but induced adipocyte LPL expression. In both the *in vivo* and *in vitro* studies, inducibility by either PPARα or PPARγ activators correlated with the tissue-specific distribution of the respective PPARs. This transcriptional induction of the LPL gene by fibrates and thiazolidinediones is mediated through binding of PPAR–RXR heterodimers to a functional PPRE sequence in the human LPL gene promoter. The regulation of LPL by fibrates and thiazolidinediones may contribute to the beneficial triglyceride lowering effect of these substances.

Results

PPARα and PPARγ activators induce LPL mRNA and activity selectively in liver and adipose tissue in vivo

Since the hypotriglyceridemic activity of fibrates and thiazolidinedione antidiabetic agents may be mediated by inducing LPL activity, we measured LPL mRNA and activity levels in various rat tissues after administration of these compounds. Organ and body weights and lipid values of rats are presented in Table I. Fenofibrate (0.5% w/w ~0.5 g/kg/day), a selective PPARα activator, lowered both serum triglyceride and cholesterol levels (Table I). In rats treated with fenofibrate, liver weight increased considerably after a 14 day treatment interval, whereas total body weight and weights of the other organs did not change significantly. LPL mRNA, which is normally undetectable in adult rat liver, was strongly induced in rat liver (Figure 1A and B), confirming our previous observations (Staels and Auwerx, 1992). In contrast, treatment with fenofibrate did not change LPL mRNA levels in adipose tissue or heart, two major sites of LPL production (Figure 1A and B). In heart and adipose tissue, no change in LPL activity was detected, whereas a strong induction of LPL activity was detected in liver (Figure 1C; $P < 0.01$). A similar tissue-selective induction of liver LPL mRNA was observed after treatment of rats with other fibrates, such as clofibrate and gemfibrozil (data not shown).

Administration of compound BRL 49653 (5 mg/kg/day for 7 days) also reduced serum triglyceride levels, but did not affect serum cholesterol concentration (Table I). Treatment with BRL 49653 resulted in a significant increase in the weight of epididymal adipose tissue from 3.5 ± 0.5 g to 5.8 ± 0.9 g (adipose tissue/body weight increased from 0.99 ± 0.13% to 1.51 ± 0.23%). Total body weight had a tendency to increase, whereas the weight of the other organs remained stable. BRL 49653 treatment resulted in a significant induction of adipose tissue LPL mRNA and activity levels (Figure 1; $P < 0.05$). In contrast, liver LPL mRNA and activity levels remained unchanged. Although heart LPL mRNA did not change, heart LPL activity levels decreased significantly (Figure 1; $P < 0.05$). This decrease in heart LPL activity was consistently observed, since in an independent experiment LPL activity decreased upon treatment with BRL 49653 (10 mg/kg/day for 7 days) from 531 ± 47 to 279 ± 39 mU/g ($n = 4$, $P < 0.05$), despite the absence of changing LPL mRNA levels (data not shown).

BRL 49653 induces LPL mRNA specifically in pre-adipocyte cells, whereas fibrates induce LPL mRNA in cells of hepatic origin

To study the cellular and molecular mechanism of this induction, we investigated the regulation of LPL gene expression by fibrates and BRL 49653 in hepatocyte and adipocyte cell lines. LPL mRNA was measured in AML-12 cells, a mouse hepatocyte cell line with a differentiated phenotype, after stimulation with fenofibric acid or BRL 49653. Fenofibric acid induced LPL mRNA at a minimal dose of 100 μM (data not shown), whereas a maximal response was seen at 500 μM (Figure 2A). Time course experiments demonstrated a maximal effect within 24 h (data not shown). In contrast, the PPARγ ligand BRL 49563 had no effect on LPL mRNA levels in these cells (Figure 2A).

To examine LPL regulation in adipocyte-like cell lines, 3T3-L1 pre-adipocyte cells were grown until confluency in medium containing fetal calf serum. Confluent cells were transferred to medium containing delipidated serum in order to maintain a more fibroblast-like phenotype, thus avoiding excessive spontaneous differentiation (Chawla and Lazar, 1994). Under these conditions, 3T3-L1 cells continue to proliferate and maintain c-*myc* gene expression,

Table I. Body weight, organ weights and plasma lipid values in rats treated with BRL 49563 or fenofibrate

	Body weight (g)	Adipose tissue weight (g)	Liver weight (g)	Triglycerides (mg/dl)	Cholesterol (mg/dl)
Control	351 ± 19	3.5 ± 0.5	14.4 ± 1.2	167 ± 19	73 ± 2
Fenofibrate (~0.5 g/kg/day)	361 ± 12	3.3 ± 0.5	19.8 ± 1.7[a]	114 ± 17[a]	47 ± 9[a]
BRL 49653 (5 mg/kg/day)	383 ± 15	5.8 ± 0.9[a]	15.5 ± 1.4	88 ± 30[a]	77 ± 14

[a]Values significantly different from control rats by Student's *t*-test ($P < 0.05$).

a marker for their proliferative state (Chawla and Lazar, 1994). Next, these cells were challenged with either fenofibric acid (500 μM) or BRL 49653 (10 μM) (Figure 2B and 2C). LPL mRNA was induced 5-fold after a 1 day treatment with BRL 49653, attaining a maximal 9-fold induction after 3 days. Fenofibric acid induced LPL mRNA levels only 3-fold after a 4 day lag period. aP2 gene expression in 3T3-L1 cells showed a parallel pattern to LPL expression after BRL 49653 or fenofibric acid treatment in 3T3-L1 cells (data not shown). The ob 1771 pre-adipocyte cell line (Negrel *et al.*, 1978) was also analyzed. Addition of BRL 49563 induced LPL mRNA levels in this cell line, whereas fenofibric acid had only a weak effect (Figure 2C).

PPARα is expressed in liver and heart, whereas PPARγ expression is restricted to adipose tissue

To further characterize the tissue-specific regulatory effects of BRL 49563 and fenofibric acid on adipose tissue and liver respectively, the expression of PPARα and PPARγ in human and rat tissues was analyzed (Figure 3A). Rat adipose tissue expressed the highest level of PPARγ. PPARγ was also expressed in human adipose tissue and in the pre-adipocyte 3T3-L1 cells, although at lower abundancy. PPARα showed a reciprocal pattern of expression, and was not detected in adipose tissue. PPARα mRNA levels were highest in rat liver, with lower levels being present in human liver as well as in the differentiated AML-12 hepatocyte cell line. Interestingly, the size of the mRNA for PPARα was substantially larger in human liver relative to its size in rat liver or the murine AML-12 cells.

We further analyzed whether PPARα and PPARγ mRNA expression was regulated by their activators and/or ligands (Figure 3B). PPARα mRNA levels did not change significantly after treatment of animals with fenofibric acid or BRL 49653 in the tissues analyzed. In contrast, adipose tissue PPARγ mRNA levels showed a tendency to increase after the addition of BRL 49653.

LPL cis-regulatory sequences mediate the induction of LPL gene expression by fibrates and thiazolidinediones

We next investigated whether LPL mRNA induction, observed *in vivo* and in the cell culture experiments, occurred at the transcriptional level. Therefore, LPL promoter constructs hooked to the chloramphenicol acetyltransferase (CAT) reporter were transiently transfected into murine 3T3-L1 cells and into the mouse hepatocyte-derived cell lines BWTG3 and AML-12. The LPL promoter deletion constructs PN-CAT, XN-CAT and AN-CAT contain the 5′ upstream regulatory sequence of the human LPL promoter from –1719 to +136, –852 to +136 and –230 to +136 bp respectively (Figure 4A). In BWTG3

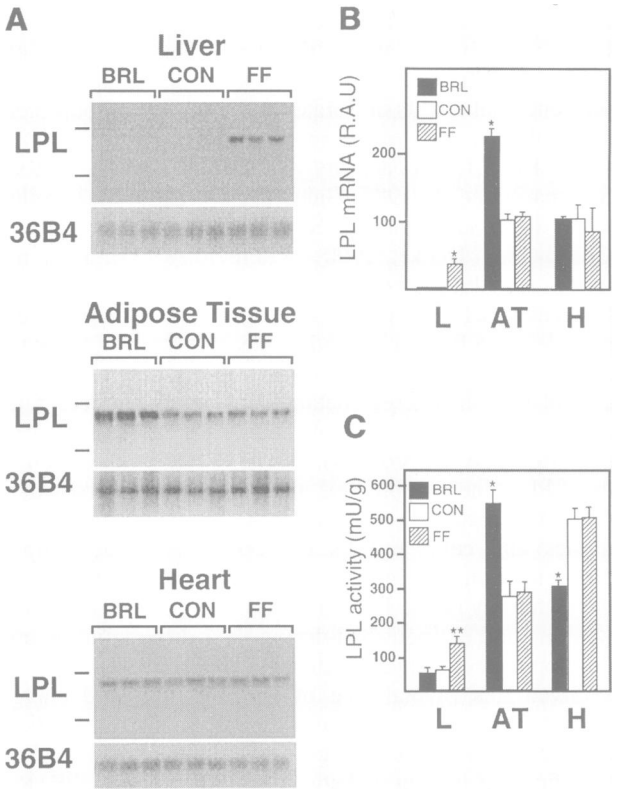

Fig. 1. Tissue-selective induction of LPL mRNA (A and B) and activity (C) in rat liver, adipose tissue and heart by fenofibrate and BRL 49653 respectively. (**A**) Expression of LPL mRNA in liver, epididymal adipose tissue and heart of animals treated with fenofibrate (FF; 0.5% w/w for 7 days, ~0.5 g/kg/day) or BRL 49653 (BRL; 5 mg/kg/day for 7 days). The blots were stripped and re-hybridized with human acidic ribosomal phosphoprotein 36B4 control cDNA. Animal treatment and preparation and analysis of RNA are described in Materials and methods. (**B**) Comparison of LPL mRNA levels in liver (L), epididymal adipose tissue (AT) and heart (H) of control rats and rats treated with fenofibrate (FF) or BRL 49653 (BRL). LPL mRNA is quantified as described in Materials and methods. Asterisks indicate values statistically significant from control by Student's *t*-test ($P < 0.05$). (**C**) LPL activity values in liver (L), epididymal adipose tissue (AT) and heart (H) of control rats and rats treated with fenofibrate (FF) or BRL 49653 (BRL). LPL activity assay is described in Materials and methods. Asterisks indicate values statistically significant from control by Student's *t*-test (*$P < 0.05$, ** $P < 0.01$).

cells, only the AN-CAT construct was activated following the addition of fenofibric acid. However, upon co-transfection with a vector that expresses mouse PPARα (pSG5-mPPARα), induction of all three reporter constructs, PN-CAT, XN-CAT and AN-CAT, independent of the presence of fenofibric acid was observed (Figure 4B). In another hepatocyte cell line, AML-12, data similar to the transfection data in BWTG3 were obtained (data not shown).

Upon co-transfection of 3T3-L1 cells with the LPL

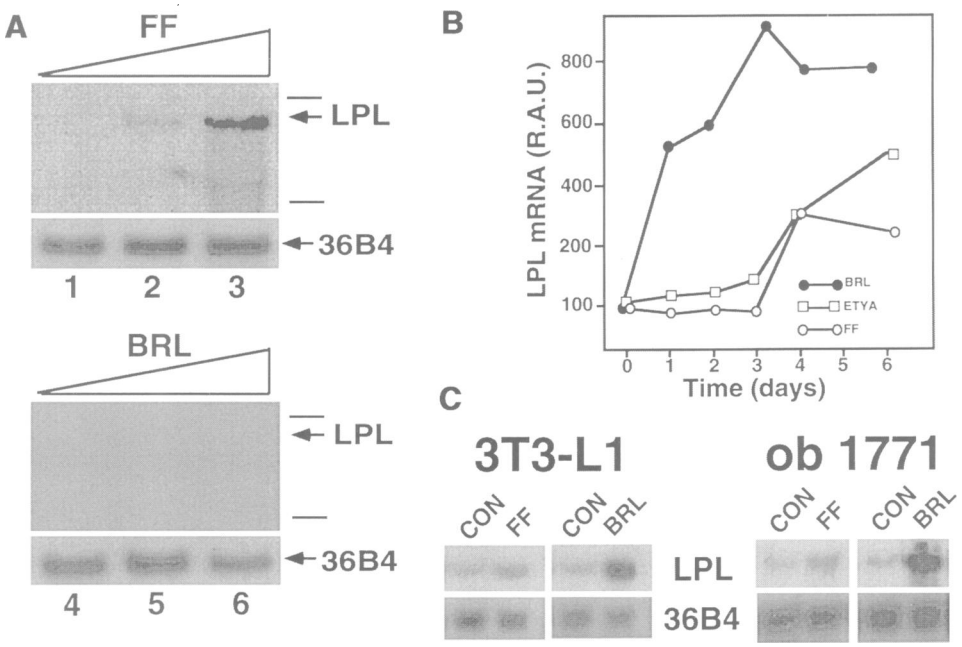

Fig. 2. Regulation of LPL mRNA expression in AML-12 hepatocytes (A), in 3T3-L1 preadipocytes (B and C) and in ob 1771 pre-adipocytes (C) by fibrates, thiazolidinediones and fatty acids. (**A**) AML-12 hepatocytes. Dose–response of LPL mRNA induction in AML-12 cells treated for 24 h with the indicated concentrations of fenofibric acid (FF; lanes 1–3, 0, 250 and 500 μM) or BRL 49653 (BRL; lanes 4–6, 0, 1 and 10 μM). A probe for 36B4 was used as a control. Cells were grown and mRNA analysis performed as described in Materials and methods. (**B**) 3T3-L1 cells. Pre-adipocytes were grown until confluency in regular medium containing fetal calf serum. Upon confluency they were changed to medium containing delipidated serum according to the protocol of Chawla *et al.* (1994). Cells were then challenged for different times with either fenofibric acid (FF; 500 μM), 5,8,11,14-eicosatetraynoic acid (ETYA; 100 μM) or BRL 49653 (BRL; 10 μM). mRNA was isolated and analyzed by dot blot hybridization. (**C**) 3T3-L1 and ob 1771 cells. 3T3-L1 cells were treated exactly as mentioned in (B). RNA isolated 3 days after treatment with either fenofibric acid or BRL 49653 was analyzed by Northern blot analysis. Undifferentiated ob 1771 cells were challenged for 3 days with either fenofibric acid (FF; 500 μM) or BRL 49653 (BRL; 10 μM).

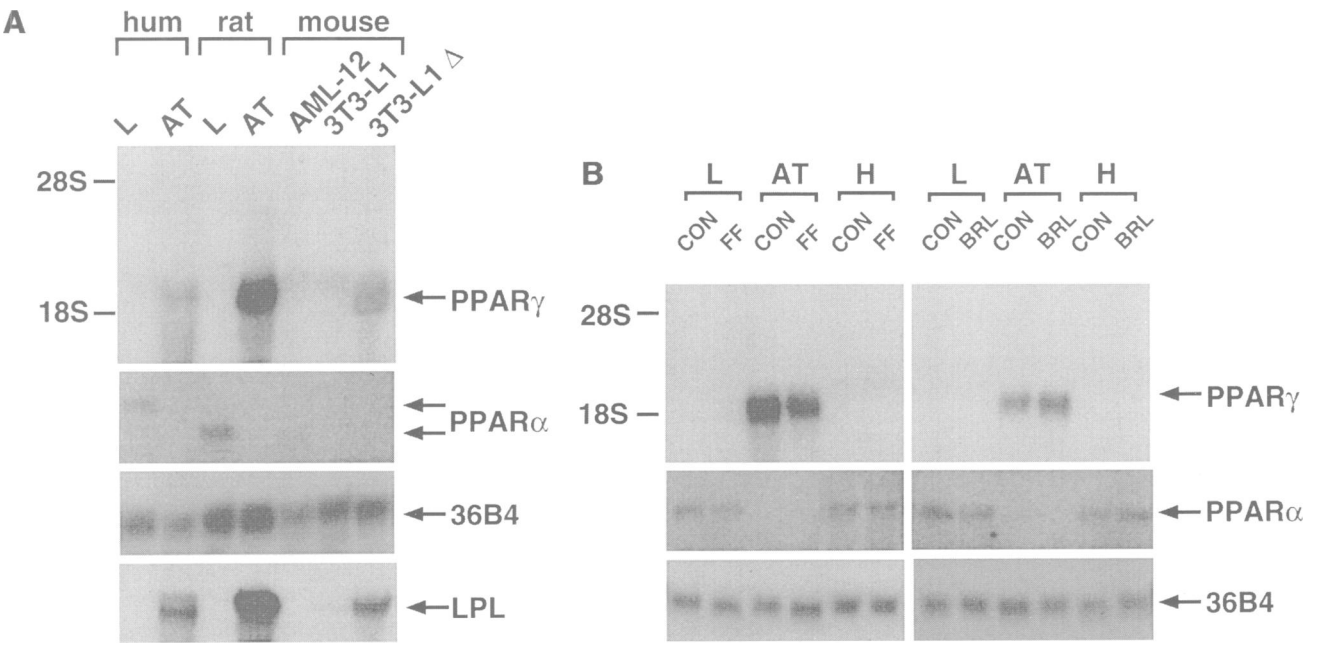

Fig. 3. Expression of the different PPAR forms in adipose tissue and liver. (**A**) Comparison of PPARα and PPARγ expression in liver and adipose tissue from rat and human and in mouse AML-12 and 3T3-L1 cell lines. The blots were re-hybridized with 36B4 and LPL cDNAs. Preparation and analysis of RNA is described in Materials and methods.The arrows indicate the various mRNA species. Note the difference in size between human and rodent PPARα mRNA. (**B**) Expression of PPARγ, PPARα and 36B4 mRNA in liver (L), epidydimal adipose tissue (AT) and heart (H) of animals treated with fenofibrate (FF; 0.5% w/w for 14 days) or BRL 49653 (BRL; 5 mg/kg/day for 7 days). Animal treatment and preparation and analysis of RNA is described in Materials and methods. The arrows indicate the various mRNA species.

promoter constructs in the presence of pSG5-haPPARγ, LPL promoter activity was also induced (Figure 4C). In this cell line, BRL 49653 alone also induced expression of the LPL promoter, indicating the presence of endogeneous PPAR. These results suggest a functional role for the various PPARs in activation of the LPL gene by BRL

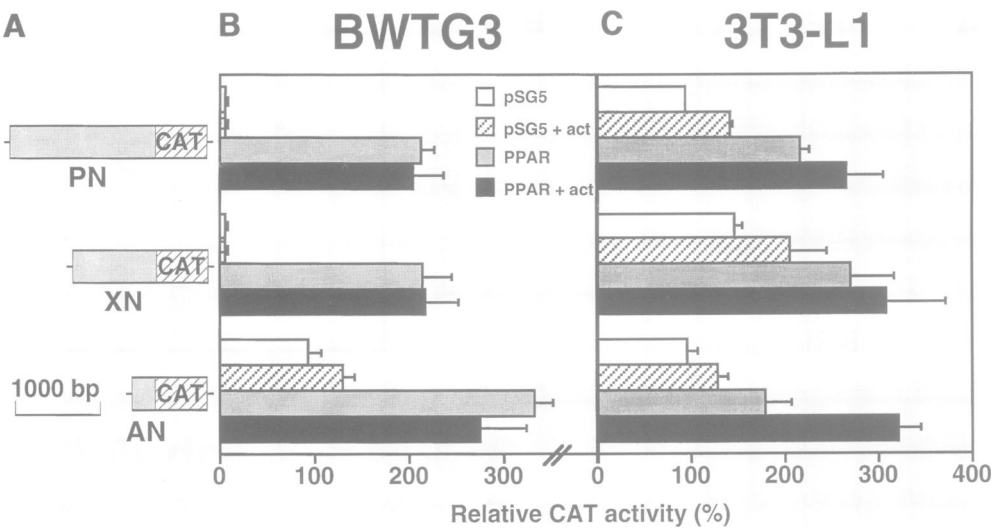

Fig. 4. Induction of the LPL gene by peroxisome proliferators is mediated by the LPL 5′-URS. (**A**) Schematic structure of the different human LPL promoter deletion constructs. (**B**) Regulation of deletions in 5′ flanking sequences of the LPL gene by fenofibric acid and/or mPPARα (PPAR) in BWTG3 mouse hepatoma cells. Cells were transfected with the LPL promoter deletion constructs, indicated in (A), either in the presence or absence of co-transfected pSG5 vector plasmid (pSG5), pSG5-mPPARα expression vector (PPAR) and/or fenofibric acid (500 μM). The results represent the mean ± SD of a triplicate experiment. The value of CAT activity observed in 3T3-L1 cells transfected with the PN-CAT construct and the pSG5 empty expression vector was arbitrarily set as 100% (see C). CAT activity was measured and expressed relative to this value. (**C**) Regulation of deletions in 5′ flanking sequences of the LPL gene by BRL 49653 (10 μM) and haPPARγ in 3T3-L1 cells. Legends are identical to Figure 2B. The results represent the mean ± SD of three individual experiments. The activity of PN-CAT in absence of any stimulus was taken as 100%.

49653 and fibrates in BWTG3, AML-12 and 3T3-L1 cells. Furthermore, they demonstrate that a *cis*-acting regulatory sequence is located within the first 230 bp upstream from the transcription initiation site which mediates this effect.

Localization of a PPRE in the LPL promoter

Results of transfections with nested deletions of the LPL promoter localized the PPRE within 230 bp upstream from the transcription initiation site of the LPL gene (AN region). In a first attempt to map the potential PPRE in the AN region, two overlapping DNA fragments, 1A and 2A (Figure 5D), each containing a different segment of the AN fragment, were PCR amplified. These fragments were labeled and tested for binding to *in vitro* translated haPPARγ and mRXRα by electrophoretic mobility shift assays (EMSA) (Figure 5A). Fragment 2A, which spans nucleotides −283 to −109, proved to be the only fragment which could specifically bind to haPPARγ and mRXRα heterodimers. Therefore, the regulatory region containing the potential PPRE is restricted to 174 bp. Subsequently, five smaller DNA fragments generated by PCR amplification encompassing different parts of the 2A fragment were synthesized and analyzed for their capacity to bind haPPARγ and mRXRα heterodimers (Figure 5B). The results narrowed the potential binding site of PPAR–RXR heterodimers to a small region located between −184 and −154 (Figure 5B and D). When haPPARγ was replaced by *Xenopus* PPARα, a similar, albeit slightly less intense, binding was observed in EMSA (data not shown). Methylation interference analysis of fragment 2F confirmed that haPPARγ–mRXRα was able to bind to this region (Figure 5C) and revealed that one methylated guanidine on the sense strand (at −168) and eight methylated guanidines on the antisense strand (from bases −167 to −157) interfered with binding of the haPPARγ–mRXRα protein complex.

An oligonucleotide containing the LPL_{wt} PPRE, as defined by physical mapping, was synthesized and used in EMSA. This oligonucleotide was capable of binding haPPARγ–mRXRα heterodimers in EMSA (Figure 6, lane 1). Similar binding data were obtained when xPPARα was used instead of haPPARγ and mRXRα was replaced by mRXRβ (data not shown), demonstrating that the LPL_{wt} PPRE element was capable of binding different types of PPAR–RXR heterodimers. Homodimers of haPPARγ or mRXRα, however, were incapable of binding to this oligonucleotide (data not shown). When increasing concentrations of unlabeled LPL_{wt} PPRE were added as competitor, binding of the haPPARγ–mRXRα heterodimer to labeled LPL_{wt} PPRE was almost completely inhibited (Figure 6A, lanes 2–5). In comparison, addition of similar concentrations of the LPL_{mut} PPRE (Figure 6A, lanes 6–9) or an unrelated unlabeled oligonucleotide (data not shown) did not result in competition. An unlabeled oligonucleotide comprising the PPRE of acyl-CoA oxidase (ACO) competed more efficiently with binding of haPPARγ–mRXRα heterodimers when compared with the LPL_{wt} PPRE oligonucleotide (Figure 6A, compare lanes 3 and 11). A second cross-competition experiment was performed, using the ACO PPRE as probe (Figure 6B). Whereas the addition of unlabeled ACO oligonucleotide (100-fold molar excess) almost completely inhibited the ability of haPPARγ–mRXRα heterodimers to bind to the ACO PPRE, addition of unlabeled LPL_{wt} PPRE induced a somewhat lower level of competition. Hence, the results of both the methylation interference assay and the EMSA experiments unequivocally demonstrate the capacity of PPAR–RXR to physically bind to the LPL PPRE localized between −169 and −157 in the human LPL promoter. This region contains two motifs related to the consensus intracellular receptor binding half-site that are arranged as direct repeats with a spacing of one nucleotide (Osumi *et al.*, 1991; Tugwood *et al.*, 1992).

The mouse LPL gene also contains a sequence TGCCCTTTCCCCT at −169 to −157 which is similar to the

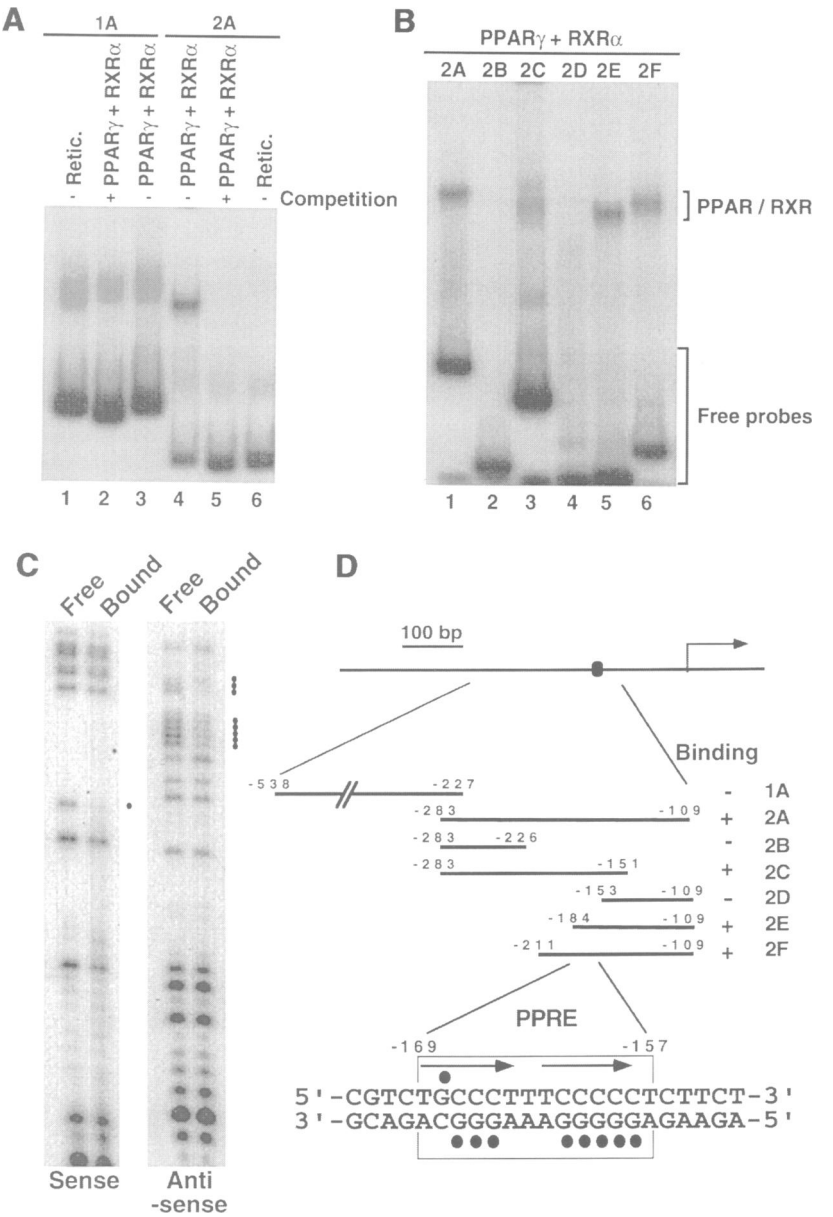

Fig. 5. Definition and mapping of a PPAR binding site in the LPL promoter. (**A**) EMSA assay was performed on PCR fragments 1A (–538 to –227) and 2A (–283 to –109) in the presence of *in vitro* translated mRXRα and haPPARγ exactly as described in Materials and methods. Competition was performed with cold 1A and 2A PCR fragments. (**B**) EMSA assay was performed on smaller PCR fragments, depicted schematically in (D), in the presence of *in vitro* translated mRXRα and haPPARγ exactly as described in Materials and methods. (**C**) Methylation interference assays were performed exactly as described in Materials and methods. The reactions using labeled sense and antisense strands are indicated. Contact interference sites of guanidine residues with the protein complex are indicated by a dot. (**D**) A scheme which depicts the LPL 5′ regulatory region and the different PCR fragments used in the EMSA in (A) and (B). In addition, a graphic depiction of the LPL PPRE and the methylated guanosines interfering with binding of the protein complex are shown in (C).

identified PPRE in the human LPL gene. In order to demonstrate that this mouse sequence could also bind haPPARγ–mRXRα heterodimers, we compared binding of these heterodimers with the mouse and human LPL_{wt} PPREs in EMSA (Figure 7). Interestingly, the mouse LPL_{wt} PPRE bound haPPARγ–mRXRα heterodimers with higher affinity (Figure 7A). The somewhat higher affinity of haPPARγ–mRXRα heterodimers for the mouse LPL PPRE was confirmed in a cross-competition experiment, where an unlabeled oligonucleotide comprising the mouse LPL_{wt} PPRE (Figure 7B, lanes 2–4) competed more efficiently with binding of haPPARγ–mRXRβ heterodimers relative to the human LPL_{wt} PPRE oligonucleotide (Figure 7B, lanes 8–10). The presence of a PPRE in the mouse LPL gene translates into a strong inducibility of the mouse LPL gene by various PPAR activators either *in vitro* in cultured mouse cell lines, such as 3T3-L1, ob 1771 and AML-12 cells (Figure 2), or *in vivo*, as demonstrated by the induction of liver LPL mRNA after treatment of mice with fenofibrate (Figure 7C; 0.5% w/w for 14 days).

The sequence identified in the LPL promoter is a functional PPRE

We next investigated whether the element identified could mediate the effects of BRL 49653, fibrates and PPAR on

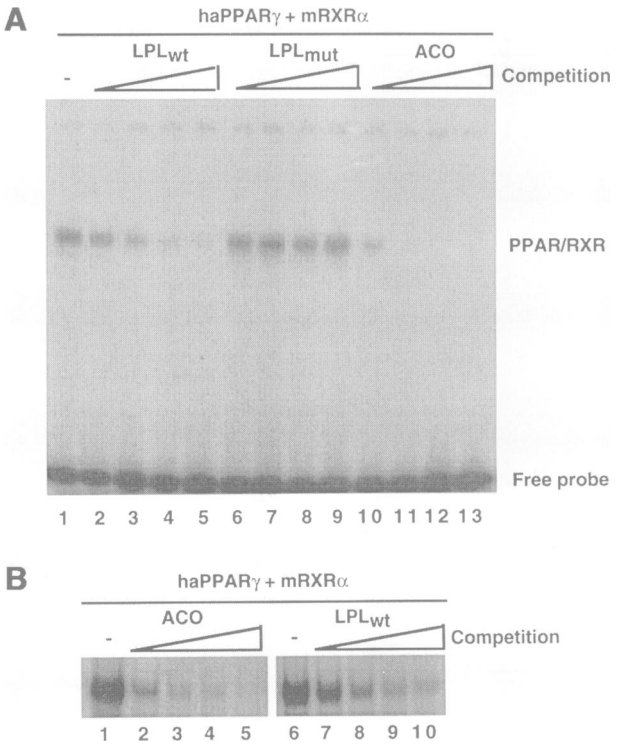

Fig. 6. PPAR–RXR heterodimers bind to the LPL PPRE. (**A**) PPAR–RXR heterodimers bind to the LPL_{wt} PPRE. EMSA was performed on end-labeled LPL_{wt} PPRE oligonucleotide in the presence of *in vitro* translated mRXRα and haPPARγ (lane 1). Competition experiments were performed in the presence of 10-, 50-, 100- or 200-fold molar excess of either unlabeled LPL_{wt} PPRE (lanes 2–5), LPL_{mut} PPRE (lanes 6–9) or ACO PPRE (lanes 10–13) oligonucleotides. (**B**) EMSA assays were performed using end-labeled ACO PPRE oligonucleotide in the presence of *in vitro* translated mRXRα and haPPARγ (lanes 1 and 6). Competition experiments were performed in the presence of 10-, 50-, 100- and 200-fold molar excess of either cold ACO PPRE (lanes 2–5) or LPL_{wt} PPRE (lanes 7–10) oligonucleotides.

transcription of the LPL gene. To this end, the wild-type PPRE in the AN-CAT construct was mutated (AN-$PPRE_{mut}$-CAT; Figure 8A). The activities of the wild-type and mutated AN-PPRE-CAT constructs were compared after transfection into 3T3-L1 cells (Figure 8B). Treatment of 3T3-L1 cells with either BRL 49653 (10 μM) or fenofibric acid (250 μM) was unable to induce expression of AN-$PPRE_{mut}$-CAT in either the presence or absence of co-transfected pSG-haPPARγ. These results are in contrast to those observed with the AN-$PPRE_{wt}$-CAT reporter, whose expression was induced by the above-mentioned activators (Figures 4 and 8). Fenofibric acid was a considerably less potent inducer of AN-CAT activity in 3T3-L1 cells than the PPARγ ligand BRL 49653. In AML-12 hepatocyte cells no induction of AN-$PPRE_{mut}$-CAT was also obtained after fenofibric acid (250 μM) and/or mPPARα co-transfection (data not shown). This was in contrast to the results obtained with the AN-$PPRE_{wt}$-CAT reporter with the same inducers.

3T3-L1 pre-adipocyte cells most likely contain low levels of PPARs (below the detection limit of Northern blot hybridization) which can mask the effects of co-transfected PPARs. Therefore, we transfected the wild-type and mutated AN-PPRE-CAT constructs into HeLa cells, which contain very low amount of the different PPARs (Figure 8C). In contrast to 3T3-L1 cells, treatment of HeLa cells with BRL 49653 (10 μM; Figure 8C) was unable to induce expression of AN-$PPRE_{wt}$-CAT. The AN-$PPRE_{wt}$-CAT reporter was, however, induced upon co-transfection of either haPPARγ by itself or the combination of haPPARγ and mRXRα. This effect was further enhanced by addition of the PPARγ ligand BRL 49653. Treatment of HeLa cells with BRL 49653 was unable to induce expression of AN-$PPRE_{mut}$-CAT in either the presence or absence of co-transfected haPPARγ.

Discussion

The distinct tissue-specific expression pattern of the different PPARs as well as their distinct activation by various activators and/or ligands suggests a highly specific function for each PPAR type. This investigation aimed to define the molecular mechanisms by which two well-established groups of synthetic PPAR activators, i.e. fibrates (considered to preferentially activate PPARα) and the anti-diabetic thiazolidinediones (PPARγ ligands), affect LPL expression in a tissue-specific manner and to investigate the relative contribution of the various PPAR subtypes to these regulatory events. We have chosen to use LPL as a target gene in these studies because a number of clinical studies suggested that the triglyceride lowering action of fibrates and polyunsaturated fatty acids is associated with an increase in LPL activity (Schonfeld *et al.*, 1982; Heller and Harvengt, 1983; Sommariva *et al.*, 1983; Gnasso *et al.*, 1986; Weisweiler, 1989; Ikeda *et al.*, 1990; Levy *et al.*, 1993). LPL is a key enzyme in the metabolism of triglyceride-rich lipoproteins and plays the role of a gatekeeper in energy metabolism by controlling the generation of fatty acids. In contrast to other genes involved in the determination of serum triglyceride levels, such as apolipoprotein C-III, which is only expressed in the liver and intestine, LPL is expressed in multiple tissues, including adipose tissue, liver, heart, mammary gland, macrophages and brain. Results obtained in this study using cultured hepatocyte and pre-adipocyte cell lines, as well as *in vivo* data obtained in rat adipose tissue or rat and mouse liver, establish that LPL mRNA and activity levels can be regulated in a tissue-specific fashion by PPARα activators and PPARγ ligands. Transient transfection assays of the LPL 5′ regulatory sequences indicated that LPL induction was mediated by a response element, located between –157 and –169 in the human LPL gene. This region contains two motifs which are somewhat related to the consensus steroid/thyroid hormone receptor half-site TGACCT, arranged as direct repeats separated by one nucleotide (DR-1), previously identified as mediating responsiveness to the PPARs in several genes involved in lipid metabolism (Osumi *et al.*, 1991; Muerhoff *et al.*, 1992; Tugwood *et al.*, 1992; Zhang *et al.*, 1992; Bardot *et al.*, 1993; Marcus *et al.*, 1993; Alvarez *et al.*, 1994; Gulick *et al.*, 1994; Palmer *et al.*, 1994; Rodriguez *et al.*, 1994; Vu-Dac *et al.*, 1994, 1995; Schoonjans *et al.*, 1995). A sequence highly comparable with the PPRE of the human LPL gene (TGCCCTTTCCCCC) (Deeb and Peng, 1989) is found in the mouse LPL gene (TGCCCTTTCCCCT) (Hua *et al.*, 1991), indicating that these rather atypical PPRE-like sequences might have an important regulatory role. We showed that PPAR–RXR heterodimers bind to the response element in the human and mouse

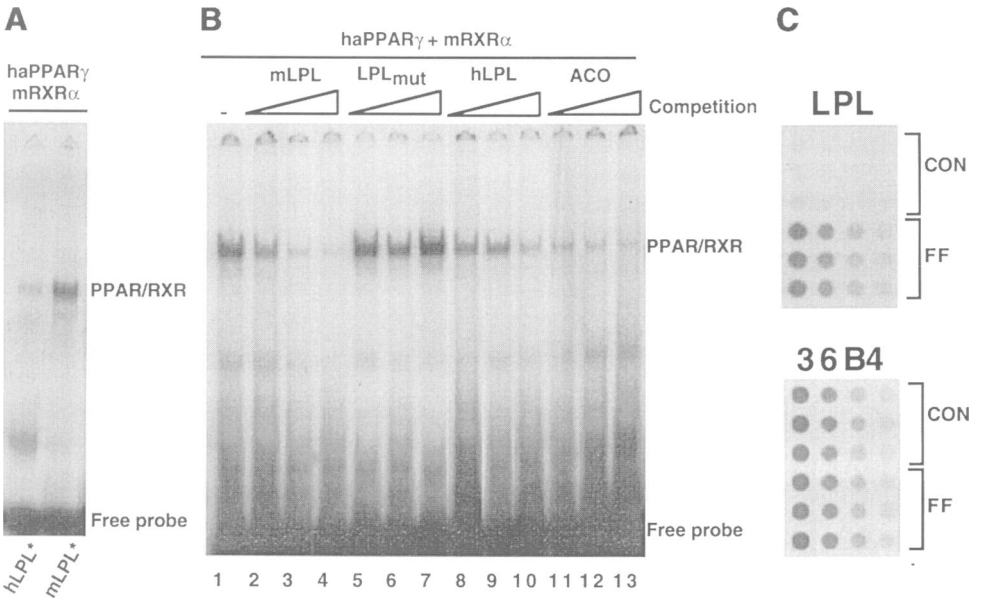

Fig. 7. PPAR–RXR heterodimers bind to the mouse LPL PPRE. (**A**) PPAR–RXR heterodimers bind with a higher affinity to the mouse LPL PPRE when compared with the human LPL PPRE. EMSA was performed on end-labeled oligonucleotides representing either the mouse or human LPL_{wt} PPREs in the presence of an identical amount of *in vitro* translated mRXRα and haPPARγ. (**B**) Competition experiments were performed in the presence of 10-, 50- and 100-fold molar excess of either unlabeled $mLPL_{wt}$ PPRE (lanes 2–4), $hLPL_{mut}$ PPRE (lanes 5–7), $hLPL_{wt}$ PPRE (lanes 8–10) or ACO PPRE (lanes 11–13) oligonucleotides and using an end-labeled $mLPL_{wt}$ PPRE oligonucleotide in the presence of *in vitro* translated mRXRα and haPPARγ (lane 1). (**C**) Expression of LPL mRNA in mouse liver of animals treated with fenofibrate (FF; 0.5% w/w for 14 days). The amount of RNA spotted was equivalent, as demonstrated by re-hybridization of the dot blot with the 36B4 control cDNA. Animal treatment and preparation and analysis of RNA are described in Materials and methods.

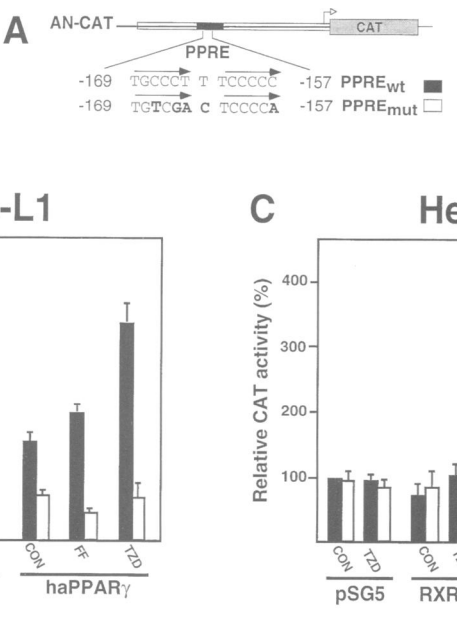

Fig. 8. Localization of a functional PPRE in the human LPL promoter. (**A**) Scheme depicting the AN-$PPRE_{wt}$-CAT and AN-$PPRE_{mut}$-CAT vectors. The bases mutated in AN-$PPRE_{mut}$-CAT are indicated in bold. (**B**) The AN-$PPRE_{wt}$-CAT (indicated in black) and AN-$PPRE_{mut}$-CAT (indicated in white) vectors were co-transfected into 3T3-L1 cells with either the pSG5 plasmid or pSG5-haPPARγ and cells were treated with 10 μM BRL 49653 (TZD), 250 μM fenofibric acid (FF) or vehicle (CON). Experiments were performed as described in Materials and methods. The results represent the mean ± SD of three independent experiments. The CAT activity of AN-$PPRE_{wt}$-CAT in the presence of co-transfected pSG5 was arbitrarily set to 100%. (**C**) The AN-$PPRE_{wt}$-CAT (indicated in black) and AN-$PPRE_{mut}$-CAT (indicated in white) vectors were co-transfected into HeLa cells with either the pSG5, pSG5-mRXRα, pSG5-haPPARγ or pSG5-mRXRα and pSG5-haPPARγ plasmids and cells were treated with 10 μM BRL 49653 (TZD) or vehicle (CON). Experiments were performed as described in Materials and methods. The results represent the mean ± SD of three independent experiments. The CAT activity of AN-$PPRE_{wt}$-CAT in the presence of co-transfected pSG5 was arbitrarily set to 100%.

LPL promoter, albeit with a weaker affinity than to the classical ACO PPRE. The LPL_{wt} PPRE is furthermore activated by PPARs, thiazolidinediones and fibric acids. The transmission of PPAR regulatory signals through such 'non-consensus' PPREs, as present in the LPL gene and as recently identified by Brun *et al.* (1996) in the aP2

gene, indicates that careful analysis of candidate PPRE-like sequences is necessary before conclusions can be drawn about their functionality.

The tissue-selective effects of the PPAR activators/ligands are highly intriguing and provide insight into their effects on triglyceride metabolism. Fibrate treatment induced LPL expression strongest in liver, whereas BRL 49653 had no effect on liver, but induced adipocyte LPL expression. These compounds had no effect on heart LPL mRNA levels. In both the *in vivo* and *in vitro* studies, inducibility of LPL mRNA expression by either PPARα or PPARγ activators correlated with the tissue-specific distribution of the respective PPARs. This selectivity of PPARα and PPARγ activators, together with the tissue-specific expression of the respective receptors, allows tissue-selective activation of the LPL gene. In this context, we need to address the minimal effects which fenofibrate exerts on both LPL mRNA levels as well as LPL promoter activity in 3T3-L1 cells. Several mechanisms, which are not mutually exclusive, can explain this observation. It is possible that the addition of fibrates to pre-adipocytes in culture results in the production of endogeneous PPARγ ligands, such as prostaglandins (Forman *et al.*, 1995; Kliewer *et al.*, 1995), which then could activate PPARγ and induce LPL gene transcription. The slower kinetics of LPL mRNA induction after fenofibrate treatment are consistent with this hypothesis. Alternatively, fibrates, such as fenofibrate (Aperlo *et al.*, 1995) or Wy 14643 (Dreyer *et al.*, 1993; Marcus *et al.*, 1993; Tontonoz *et al.*, 1994a,b; Kliewer *et al.*, 1995; Lehmann *et al.*, 1995; Yu *et al.*, 1995), are reported to activate PPARγ, although to a lower extent than PPARγ ligands. Identification of the natural ligands for all PPAR types, as well as the identification of high affinity synthetic agonists and antagonists, will most likely result in chemicals which will be more selective than the currently available compounds, allowing target gene activation in a specific tissue of interest while limiting undesired side-effects in other tissues. These compounds might have interesting therapeutic features, especially if one considers the context of the widespread use of fibrates in the treatment of diet-resistant hyperlipidemia and the potential usefulness of thiazolidinediones in treatment of non-insulin-dependent diabetes. In addition to studies aimed at defining new agonists and antagonists, it is of equal importance to understand the distinct structural features of the ligand binding domain of the PPAR subtypes, which allow similar and related receptors to be activated with a high degree of specificity by certain ligands or activators.

The paradoxical relationship existing between LPL mRNA (Figure 1A and B) and activity (Figure 1C) in the heart after treatment with BRL is very puzzling. In view of the constant levels of LPL mRNA, it is relatively unlikely that this reduction in LPL activity is mediated by a change in LPL gene transcription induced by PPARγ. Furthermore, with Northern blot analysis no PPARγ mRNA was detected in rat heart tissue, making it unlikely that PPARγ is a major player, although the presence of minute quantities of PPARγ mRNA, which might be sufficient to mediate this effect, might be overlooked by this technique. We believe, however, that it is more likely that BRL 49653 has other effects beyond the well-established activation of PPARγ. Recent studies suggest

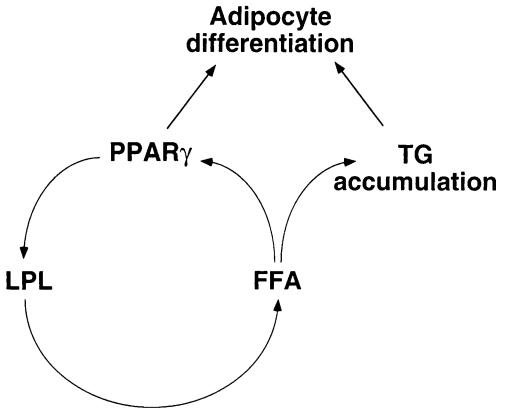

Fig. 9. Schematic representation of the interdependent role of LPL and PPARγ in adipocyte differentiation.

that several signal transduction pathways are affected by thiazolidinediones, most notably protein tyrosine phosphatase activity (Maegawa *et al.*, 1995) and calcium fluxes (Buchanan *et al.*, 1995). Further studies are, however, necessary to delineate which signaling pathway is responsible for this observed discrepancy.

The demonstration of a PPRE in the LPL gene has important implications for adipocyte physiology. The adipose-restricted PPARγ2 type can promote pre-adipocyte determination as well as terminal differentiation (Tontonoz *et al.*, 1994a,b). In fact, PPARγ2 mRNA is itself induced in the earliest steps of adipocyte differentiation before the induction of early marker genes such as LPL. Many of these genes induced during adipocyte differentiation encode proteins involved in lipid storage and metabolism. The PPAR-mediated induction of LPL expression hence explains the mechanism by which the differentiation-linked induction of LPL occurs. The observation that the LPL gene appears to be slightly more efficiently activated by PPARγ than by PPARα (data not shown) is consistent with the specific role of PPARγ in adipose differentiation (Tontonoz *et al.*, 1994b; Brun *et al.*, 1996). The increase in lipolytic capacity as a result of induction of LPL by PPARγ will result in increased delivery of fatty acids to the adipocytes. This points to the possible existence of a positive regulatory feedback loop involving PPARγ and LPL and aim at promoting and maintaining the mature adipocyte phenotype (Figure 9). In fact, it was recently reported that in addition to the thiazolidinediones, certain fatty acid-derived prostaglandin derivatives, whose delivery to the cell is increased by LPL, bind to and/or activate PPARγ (Forman *et al.*, 1995; Kliewer *et al.*, 1995; Lehmann *et al.*, 1995; Yu *et al.*, 1995). This hypothesis is supported by the observation that fatty acids (including arachidonic acid-derived prostaglandins) and fatty acid analogs induce expression of adipocyte-specific genes and enhance adipocyte conversion (Gaillard *et al.*, 1989; Amri *et al.*, 1991; Distel *et al.*, 1992; Chawla and Lazar, 1994; Tontonoz *et al.*, 1994a,b). In addition to being potent PPAR activators (Gottlicher *et al.*, 1992; Keller *et al.*, 1993; Kliewer *et al.*, 1994; Forman *et al.*, 1995; Yu *et al.*, 1995), LPL-derived fatty acids will provide the necessary building blocks for triglyceride accumulation, ultimately enhancing adipocyte differentiation. The PPAR-mediated activation of LPL expression in cells of the adipogenic

lineage might furthermore be responsible for the previously reported capacities of thiazolidinediones to induce adipocyte differentiation and induce the development of obesity (Hiragun *et al.*, 1988; Ikeda *et al.*, 1990; Sparks *et al.*, 1991; Kletzien *et al.*, 1992; Sandouk *et al.*, 1993; Batchvarova *et al.*, 1995; Hu *et al.*, 1995; Martin *et al.*, 1995; Teboul *et al.*, 1995; Brun *et al.*, 1996). In this context, it is interesting to note that the PPARγ-mediated effects of BRL 49653 on LPL expression are acting in concert with reduced *ob* mRNA and leptin levels and the associated increase in caloric uptake enhancing energy storage in the adipocytes observed with this compound (De Vos *et al.*, 1996; Zhang *et al.*, 1996).

One remaining question is the relationship between PPARγ, thiazolidinediones and insulin resistance. It is tempting to speculate that the increase in LPL activity in adipose tissue is related to the antidiabetic effects of the thiazolidinediones. Due to the enhanced triglyceride clearance in adipose tissue, less triglycerides will become available to be hydrolyzed to fatty acids in the vascular bed of the muscle. In view of the inhibitory effects of fatty acids on insulin-mediated glucose metabolism (Randle *et al.*, 1961), the decrease in fatty acids delivered to the muscle cells might be responsible for the improvement in insulin sensitivity of this tissue. The apparent inverse relationship between certain adipose tissue depots and insulin sensitivity is another argument supporting the relation between adipose tissue, fatty acids and insulin resistance. In fact, in contrast to the improvement in insulin resistance seen with thiazolidinediones, which have a tendency to increase the adipose tissue mass, lipodystrophic syndromes, characterized by the absence or a decrease in lipid storage in adipocytes, are characterized by extreme insulin resistance (Moller and Flier, 1991; Flier, 1995).

The physiological importance of the induction of LPL mRNA and activity in adult liver and in hepatic cells is unclear at present. LPL activity in the liver can be involved in the clearance of triglyceride-rich lipoprotein particles, a phenomenon stimulated by fibrates (reviewed in Auwerx *et al.*, 1992). Furthermore, the induction of LPL in the liver may facilitate peroxisome proliferation, a well-known phenomenon in rodents (Lock *et al.*, 1989; Osmundsen *et al.*, 1991). In fact, LPL will generate fatty acids which are taken up by the hepatocyte and might overwhelm its capacity for β-oxidation, ultimately enhancing fibrate-induced peroxisome proliferation. However, it is becoming increasingly evident that PPARs play an important role not only in the control of intracellular lipid metabolism and β-oxidation, but also in a wide range of activities, such as lipid transport and lipoprotein physiology. We have previously shown that PPARs control the expression of both human apoA-I and apoA-II, two major protein components of HDL (Vu-Dac *et al.*, 1994, 1995). Fibrates, thiazolidinediones, fatty acids and PPARs may also influence the metabolic fate of triglyceride-rich apoB-containing particles by stimulating LPL expression. This stimulation of LPL production in liver by fibrates occurs in addition to the decrease in hepatic production of apoC-III, which by itself has a beneficial effect on plasma triglyceride levels (Hertz *et al.*, 1995; Staels *et al.*, 1995). Indeed, due to the reduced apoC-III concentration, both LPL-mediated lipolysis (Wang *et al.*, 1985) and receptor-mediated clearance of triglyceride-rich particles (Aalto-Setälä *et al.*, 1992; de Silva *et al.*, 1994) will be improved, resulting in lowering of plasma triglyceride levels.

In conclusion, LPL mRNA and activity levels can be regulated in a tissue-specific fashion by PPARα activators and PPARγ ligands. The induction of LPL mRNA by these compounds is mediated by the transcription factor PPAR, which interacts with a PPRE in the LPL promoter. In adipose tissue, the increase in LPL production after treatment with thiazolidinediones will enhance the clearance of plasma triglycerides and provide the (pre-)adipocytes with additional fatty acids, which can further stimulate the transactivation capacity of PPAR or which can be stored in the form of triglycerides. In the liver, enhanced production of LPL, together with reduced production of apoCIII (Staels *et al.*, 1995), may contribute to the hypolipidemic action of these compounds. This tissue-selective induction of LPL gene transcription by activators of different PPARs demonstrates the feasibility of the development of highly specific PPAR subtype-specific agonists and antagonists, which can be used as drugs.

Materials and methods

Materials

BRL 49653 and fenofibric acid were kind gifts of Dr Larry Hamann (Ligand Pharmaceuticals, San Diego, CA) and Dr Alan Edgar (Laboratoires Fournier, Daix, France).

Recombinant plasmids

The construction of the LPL promoter-containing CAT vectors PN (−1719 to +136), XN (−852 to +136) and AN (−230 to +136) was described elsewhere (Schoonjans *et al.*, 1993a). Site-directed mutagenesis of the LPL PPRE was accomplished by introduction of a *Sal*I restriction site into AN-CAT. Briefly, two restriction site-containing PCR fragments, *Hin*dIII-5′AN $PPRE_{1/2}$-*Sal*I (PCR 1) and *Sal*I-$PPRE_{1/2}$ AN3′-*Bam*HI (PCR 2), were synthesized using the primers 5′-AATAAGCTTCCGGGT-AGAGTGGAACCCCTT-3′ and 5′-GGAGTCGACAGACGGAAAAA-TTTGCTTTGTT-3′ (PCR 1) and primers 5′-TCTGTCGACTCCCAC-TCTTCTCGTTGGCAG-3′ and 5′-AATGGATCCGGCTGAGCCGG-AGGCTGGAGA-3′ (PCR 2) and subcloned into pBLCAT5 (Luckow and Schütz, 1987). The expression vectors pSG5-haPPARγ (Aperlo *et al.*, 1995), pSG5-mPPARα (Isseman and Green, 1990), pSG5-mRXRα (Leid *et al.*, 1992) and pSG5-mRXRβ (Leid *et al.*, 1992) were described previously.

Animal studies

Adult (95-days old) Sprague–Dawley rats were housed in groups and acclimated to a 12:12 h day/night illumination cycle (light from 8 a.m. to 8 p.m.). Rats were divided into groups of a minimum of three animals each and were treated for either 7 or 14 days. The first group received BRL 49653 (5 mg/kg/day) by gavage. The second group of animals received 0.5% w/w fenofibrate (~0.5 g/kg/day) mixed with their food, whereas the third group of animals served as controls and received 10% carboxymethylcellulose (vehicle for gavage) by gavage. In a separate experiment, adult C57Bl6 male mice were fed for 14 days with either a control chow ($n = 3$) or the same chow containing 0.5% w/w fenofibrate. At the end of the treatment period all animals were weighed and sacrificed by exsanguination under ether anesthesia between 8.00 and 10.00 a.m. Epididymal adipose tissue (in rats) and liver (in rats and mice) were removed, weighed, rinsed with 0.9% NaCl and frozen in liquid nitrogen until RNA preparation.

Tissue biopsies

Omental adipose tissue was obtained from non-obese adult subjects undergoing elective surgery. All subjects had fasted overnight before surgery (between 8.00 and 10 a.m.) and received i.v. saline infusion. They had given informed consent and the project was approved by the Ethics Committee of the Institut Pasteur of Lille. Adipose tissue was immediately frozen in liquid nitrogen until RNA preparation. Human

liver specimens were collected from physically healthy multiorgan donors for transplantation who died after severe traumatic brain injury. Permission to use the remaining, non-transplanted part of donor liver for scientific research was obtained from the Ethics Committee.

Cell culture and transient transfection assays

The mouse hepatoma and pre-adipocyte cell lines, BWTG3 (Schoonjans *et al.*, 1993a), ob 1771 (Negrel *et al.*, 1978) and 3T3-L1 (ATCC), were maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with 10% delipidated and charcoal-treated fetal calf serum (FCS), L-glutamine and antibiotics, unless stated otherwise. AML-12 mouse hepatocytes (Wu *et al.*, 1994) were maintained in DMEM/Ham's F-12 supplemented with 10% delipidated and charcoal-treated FCS, insulin, transferrin and selenium (ITS; Collaborative Research), dexamethasone (0.1 μM) and gentamycin (50 μg/ml). Fenofibric acid, BRL 49563 (both in DMSO) and fatty acids (in ethanol) were added to the medium at the appropriate concentrations and times indicated. Control cells received vehicle only. Fatty acids were complexed to serum albumin contained in delipidated and charcoal-treated FCS by preincubation for 45 min at 37°C. Transfections and CAT assays were carried out exactly as described previously (Vu-Dac *et al.*, 1994).

RNA analysis

RNA preparation, Northern blot hybridizations and quantification of total cellular RNA were performed as described previously (Staels and Auwerx, 1992). A human LPL cDNA (Auwerx *et al.*, 1989), mPPARα (Isseman and Green, 1990), haPPARγ (Aperlo *et al.*, 1995) and human acidic ribosomal phosphoprotein 36B4 (Masiakowski *et al.*, 1982) were used as probes.

Measurements of plasma lipids and LPL activity

Plasma lipids (cholesterol and triglycerides) were measured colorimetrically (490 nm) using a microtiter plate reader and reagents from Bio-Merieux (Marcy-l'Etoile, France). LPL activity was measured in tissue extracts according to the procedure of Ramirez (Ramirez *et al.*, 1985). One unit of enzyme activity was defined as the amount of enzyme that releases 1 μmol oleate/min at 25°C.

Electrophoretic mobility shift assays (EMSA) and oligonucleotide sequences

haPPARγ (Aperlo *et al.*, 1995), xPPARα (Dreyer *et al.*, 1992), mRXRα (Leid *et al.*, 1992) and mRXRβ (Leid *et al.*, 1992) proteins were synthesized *in vitro* in rabbit reticulocyte lysate (Promega). Molecular weights and quality of the *in vitro* translated proteins were verified by SDS–PAGE. PPAR (2 μl) and/or RXR (2 μl) were incubated for 15 min on ice in a total volume of 20 μl with 1 ng probe, 2.5 μg poly(dI·dC) and 1 μg herring sperm DNA in binding buffer (10 mM Tris–HCl, pH 7.9, 40 mM KCl, 10% glycerol, 0.05% Nonidet P-40 and 1 mM DTT). For competition experiments, increasing amounts (from 10- to 200-fold molar excess) of cold oligonucleotide ACO PPRE (Tugwood *et al.*, 1992) (5′-GATCCCGAACGTGACCTTTGTCCTGGTCCC-3′), hLPL-PPRE (5′-GATCCGTCTGCCCTTTCCCCCTCTTCA-3′), mLPL-PPRE (5′-GATCCGTCTGCCCTTTCCCCTTCTTCA-3′) and/or $hLPL_{mut}$-LPL (5′-GATCCGTCTGCCATTTCCCACTCTTCA-3′) were included just before adding T4 PNK end-labeled oligonucleotide or PCR fragment. DNA–protein complexes were separated by electrophoresis on a 4% polyacrylamide gel in 0.25× TBE buffer at 4°C (Fried and Crothers, 1983). PCR fragments 1A (–538 to –227) and 2A (–304 to –109) were synthesized using sense and antisense primers 5′-TCG-AGCTTTTGTCCATCACATAAGC-3′, 5′-CGCGGATCGATACCCGG-GGGCCCCCTGTCTAAG-3′ and 5′-TCGAGAAAACAGGTGATTGT-TGAGT-3′, 5′-TCGAGAACGTTTGAGCAAACA-3′ respectively. PCR fragments 2B and 2C were synthesized using the 2A sense primer and antisense primers 1A and 5′-GAAGAGGGGGAAAGGGCAGAC-3′ respectively. PCR fragments 2D, 2E and 2F were synthesized using the sense primers 5′-GATCCTCTCGTTGGCAGGGTTGATCCT-3′, 5′-GCAAATTTTTCCTCTAGAGTCTGCCCTTTC-3′ and 5′-TAAGCTA-AGCTCTAGAACAGGAGCCT-3′ respectively and the 2C antisense primer.

Methylation interference assay

PCR fragments overlapping the –211/–109 LPL promoter region were [γ-^{32}P]ATP end-labeled either on the sense or the antisense strand. After partial methylation of DNA probes, a 5-fold up-scaled preparative EMSA assay was performed as described above. The protein-bound and protein-free bands were cut from the gel and probes were electrophoresed on a DEAE membrane, eluted and precipitated. Purified probes were treated with 1 M piperidine at 95°C for 30 min. Piperidine was removed by evaporation and DNA samples were dissolved in formamide and electrophoresed on a 8% acrylamide–7 M urea sequencing gel in 1× TBE.

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