Peroxisome Proliferator-activated Receptor α Activates Transcription of the Brown Fat Uncoupling Protein-1 Gene

A LINK BETWEEN REGULATION OF THE THERMOGENIC AND LIPID OXIDATION PATHWAYS IN THE BROWN FAT CELL*

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High expression of the peroxisome proliferator-activated receptor α (PPARα) differentiates brown fat from white, and is related to its high capacity of lipid oxidation. We analyzed the effects of PPARα activation on expression of the brown fat-specific uncoupling protein-1 (ucp-1) gene. Activators of PPARα increased UCP-1 mRNA levels severalfold both in primary brown adipocytes and in brown fat in vivo. Transient transfection assays indicated that the (−4551)/UCP1-CAT construct, containing the 5′-regulatory region of the rat ucp-1 gene, was activated by PPARα co-transfection in a dose-dependent manner and this activation was potentiated by Wy 14,643 and retinoid X receptor α. The coactivators CBP and PPARγ-coactivator-1 (PGC-1), which is highly expressed in brown fat, also enhanced the PPARα-dependent regulation of the ucp-1 gene. Deletion and point-mutation mapping analysis indicated that the PPARα-responsive element was located in the upstream enhancer region of the ucp-1 gene. This −2485/−2458 element bound PPARα and PPARγ from brown fat nuclei. Moreover, this element behaved as a promiscuous responsive site to either PPARα or PPARγ activation, and we propose that it mediates ucp-1 gene up-regulation associated with adipogenic differentiation (via PPARγ) or in coordination with gene expression for the fatty acid oxidation machinery required for active thermogenesis (via PPARα).

The peroxisome proliferator-activated receptor α (PPARα) is a fatty acid-activated transcription factor that plays a key role in the transcriptional regulation of genes involved in cellular lipid metabolism (1). PPARα together with PPARγ and PPARβ/δ belong to a subgroup of the nuclear hormone receptor superfamily that heterodimerizes with the 9-cis-retinoic acid receptors (RXRs) (2–5). The PPAR-RXR heterodimer binds to specific response elements (PPREs), which consist of a direct repeat of the consensus half-site motif spaced by one nucleotide (DR-1) (6). Fatty acids, peroxisome proliferators, and fibrate hypolipidemic drugs can activate PPARα (1, 4), and natural (leukotriene B4) or synthetic (fibrate Wy 14,643) specific ligands for PPARα have been identified (7). In contrast, 15-deoxy-D12,14-prostaglandin J2 and thiazolidinedione antidiabetic agents are selective ligands for PPARγ (8–10). In addition to ligand selectivity, PPAR subtypes have been involved in different biological functions. PPARα is mostly expressed in tissues with high rates of fatty acid oxidation and peroxisomal metabolism, such as brown fat, liver, or heart (1, 11). Recent studies of PPARα-null mice have confirmed that PPARα is necessary in vivo for hepatic fatty acid oxidation and ketone body synthesis during starvation (12). PPARγ, which is ubiquitously expressed, seems to be involved in basic lipid metabolism (11). High expression of PPARγ is mainly restricted to white (WAT) and brown (BAT) adipose tissue (13). Hence, in contrast to the role of PPARα in cellular lipid catabolism, PPARγ regulates adipogenesis (i.e. lipid deposition) (13, 14).

BAT is a major site for nonshivering thermogenesis in mammals. Its thermogenic capacity relies on the presence of an inner mitochondrial protein uniquely expressed in brown adipocytes, the uncoupling protein (UCP) (15), now referred to as UCP-1 since the discovery of the more widely expressed UCP-2 and UCP-3 (for review, see Ref. 16). Brown fat thermogenesis is mainly controlled by norepinephrine released from sympathetic terminals innervating the tissue, although nuclear receptor-mediated pathways have also been described. Thus, activation of PPARγ promotes HIB-1B brown adipocyte differentiation (17), and up-regulates ucp-1 gene expression (18). Furthermore, we demonstrated that retinoic acid is a powerful inducer of ucp-1 gene transcription, acting through retinoic acid receptors and RXRs (19, 20). The 5′-flanking region of the rat ucp-1 gene contains the proximal regulatory promoter, including C/EBP-regulated sites (21) and the main cAMP-regulatory element (22), and an upstream enhancer involved in complex regulation by retinoic acid receptors, RXR, and thyroid hormone nuclear receptors (19, 20, 23). A site responsive to PPARγ activators has also been located in the upstream enhancer of the murine ucp-1 gene (18).

BAT highly coexpresses not only PPARγ and PPARδ subtypes but also PPARα (24). BAT stores triglycerides but, in contrast to WAT, it uses lipids as oxidative substrates to generate heat. Since PPARα induces the expression of fatty acid oxidation enzymes in tissues other than BAT (6), it may do so in BAT in association with thermogenic requirements. Here we
report that PPARα activators induce ucp-1 gene expression in brown adipocytes and in BAT in vivo, acting through a PPRE located in the upstream enhancer of the ucp-1 gene that is also responsible for PPARα-dependent regulation. PPARα is proposed to coordinate the activation of lipid oxidation and thermogenic activity in brown fat.

**EXPERIMENTAL PROCEDURES**

**Materials**—Wy 14,643 (pirimic acid) and 15-deoxy-Δ12,14-prostaglandin J3 were obtained from Cayman Chemicals. Troglatizone and BRL 49653 were kind gifts from Dr. T. Leff (Parke-Davis Research) and Dr. L. Castella (Gallou (France), respectively). Clofibrate, bezafibrate, Ly171883, 3,5,3′-triodothyronine, insulin, norepinephrine, and 8-bromo-cAMP were obtained from Sigma. Methoprene was from Promochem.

**Cell Culture**—Primary culture of differentiated brown adipocytes was performed as described previously (19), and grown in 5 ml of Dulbecco's modified Eagle's medium-Ham's F-12 medium (1:1) supplemented with 10% fetal calf serum, 20 nM insulin, 2 nM 3,5,3′-triiodothyronine, and 100 μM ascorbate. Experiments were performed on day 9 of culture when 80–90% of the cells were considered to be differentiated on the basis of lipid accumulation and acquisition of brown adipocyte morphology. Brown adipocytes were exposed to 10 μM Wy 14,643 for 24 h or at the concentrations and times indicated in the experiments. Cell pellets were exposed to various PPAR agonists for 24 h, except for 15-deoxy-Δ12,14-prostaglandin J3, which was added at a final concentration of 10 μM for 6 h. As indicated, cycloheximide (Sigma) was used at a dose of 5 μg/ml as reported (19).

HepG2 human hepatoma cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. The HIB-1B brown adipocyte cell line, kindly provided by Dr. B. Spiegelman, was cultured out in a final volume of 20 μl containing 10 nm Tris-HCl (pH 8.0), 0.05% Nonidet P-40, 1 nm dithiothreitol, 40 nm KCl, 6% glycerol, and 2 μg of poly(dI)·(dC). Samples were analyzed by electrophoresis at 4 °C for 60–80 min in nondenaturing 5% polyacrylamide gels in 0.5 TBE (44.5 mM Tris, 44.5 mM borate, 1 mM EDTA). In the competition experiments, 100-fold molar excess of unlabeled oligonucleotide was included in each respective binding reaction. When indicated, 1 μl of antiserum against PPARα (N-19), PPARγ (N-20), RXRα (n-20), or ETS (C-275) from Santa Cruz was used.

**RESULTS**

**Activators of PPARα Induce the Expression of the ucp-1 Gene in Differentiated Brown Adipocytes**—To analyze whether PPARα agonists modulate the expression of the ucp-1 gene, primary cultures of murine brown adipocytes were used since they express all three PPAR subtypes (24). As shown in Fig. 1, expression of brown adipocytes differentiated in culture (day 9) to PPARα activators resulted in a 2-fold (15-deoxy-Δ12,14-prostaglandin J3) to 8-fold (10 μM BRL 49653) increase in UCP-1 mRNA levels. When PPARα activators, such as several flurbiprofen and the PPARα-specific ligand Wy 14,643, were tested an even higher (3–12-fold) increase in UCP-1 mRNA expression was detected. In contrast, COX mRNA expression did not respond to PPAR activators, thus indicating that the effect of PPAR activators is specific for UCP-1 mRNA.

Exposure to Wy 14,643 led to a time-dependent increase in UCP-1 expression (Fig. 2A) and maximum induction was attained at 10 μM after 12 h of exposure to the PPARα ligand, and maintained after 24 h (Fig. 2B). The maximal effect of 10 μM Wy 14,643 on UCP-1 mRNA levels resulted in an induction that was around 40% that of 0.5 μm norepinephrine (20-fold ±
able cAMP derivative (4.3-fold)

Brown adipocytes differentiated in culture from stromal vascular cells (day 9) were exposed for 24 h (except 15-deoxy-

Northern blot. Points are means from three independent experiments

Bars are means from two to three independent experiments on different cultures and S.E. is indicated when n = 3. An example of the Northern blot analysis is depicted in the bottom of the figure. Arrows indicate the position of the two UCP-1 mRNA species in mice (1.6 and 1.9 kb) and the mitochondrial genome-encoded COII mRNA (0.8 kb), which was used as a control.

Fig. 3. Effects of 10 µM Wy 14,643 in the presence of cycloheximide, an inhibitor of protein synthesis (Fig. 3A). Cycloheximide treatment led to lower basal expression of UCP-1 mRNA, as already described (19), but it did not affect the ability of Wy 14,643 to increase UCP-1 mRNA.

When the effects of the RXR-specific agonist methoprene were analyzed (Fig. 3B), results showed that besides its reported direct action upon UCP-1 mRNA expression (20), there was a synergistic effect when both the PPARα and the RXR ligands were added, suggesting a PPARα-RXR heterodimer-mediated effect on ucp-1 gene expression.

PPARα Induces the Rat ucp-1 Gene Promoter Activity—Primary brown adipocytes were transiently transfected with a plasmid containing the upstream 4.5 kb of the rat ucp-1 gene fused to a CAT reporter gene. As shown in Fig. 4, PPARα activators increased the (~4551)UCP1-CAT activity at least 2-fold, in the same range of the effect caused by BRL 49653. Responsiveness of (~4551)UCP1-CAT to PPARα activators was enhanced 6-fold by co-transfection of the expression vector for PPARα. Thus, expression of both endogenous ucp-1 gene and transfected ucp-1 gene promoter are up-regulated by PPARα activators in primary brown adipocytes.

RXRa Enhances the PPARα-dependent Induction of the ucp-1 Gene Promoter—To further investigate the transcriptional regulation by PPARα of the ucp-1 gene promoter, we used the brown adipocyte-derived HIB-1B cell line. These cells express PPARγ and PPARδ but not PPARα (24). Thus, HIB-1B cells provide a useful model of brown fat-derived cell in which PPARα-dependent regulation rely on transfectected receptor. In agreement, Wy 14,643 did not modify (~4551)UCP1-CAT activity (Fig. 5A). However, co-transfection of pSG5-PPARα induced (~4551)UCP1-CAT activity 3-fold in the absence and nearly 7-fold in the presence of 10 µM Wy 14,643. Co-transfection of pRSV-RXRa caused a synergistic increase in the PPARα-dependent effect upon (~4551)UCP1-CAT activity. We next performed co-transfection experiments using HepG2 cells to avoid any interference of PPARγ in the observed effects. The HepG2 cell line was chosen because, in contrast to HIB-1B cells, does not express PPARγ nor PPARα (31), and has been widely used to analyze PPARγ regulation of gene transcription (31–33). As shown in Fig. 5B, co-transfection of pSG5-PPARα enhanced (~4551)UCP1-CAT activity and its responsiveness to Wy 14,643 in a dose-dependent manner, and maximal effects were observed at 0.3 µg of pSG5-PPARα. This amount of vector was the same at which the maximum synergistic enhancement by
When co-transfected together with pSG5-PPARα (−4551)UCP1-CAT activity was 5- and 7-fold, respectively (Fig. 6). Transfection of pCMX-CBP or pSV-PGC1 alone enhanced basal a responsive region of the rat element in the upstream region of the ucp-1 gene Enhanc-er—region of the rat gene promoter indicated the presence of a direct repeat with 1-base pair spacing related to a consensus PPRE (34) (Fig. 8A, arrows indicate half-site-related motifs). This −2485/−2458 sequence (UCP1-PPRE) in the rat ucp-1 gene promoter is highly conserved when compared with the previously reported PPARγ-responsive element in the murine ucp-1 gene (18) and to the corresponding sequence in the human ucp-1 gene promoter (35) (Fig. 8A). Electrophoretic gel mobility shift assays were performed using the UCP1-PPRE as labeled probe. As shown in Fig. 8B, in vitro transcribed/translated RXRα alone (lane 2) did not bind significantly to this sequence although two nonspecific bands were detected as with the reticulocyte lysate (lane 1, n.s.). incubation with a mixture of PPARα or PPARγ with RXRs resulted in the formation of the respective heterodimer complexes (lanes 3 and 4, respectively). To further assess the interaction of UCP1-PPRE with PPARα-RXRα or PPARγ-RXRα heterodimers found in nuclear extracts from differentiated brown adipocytes in primary culture (Fig. 8C) or from BAT (Fig. 8D), supershift assays were performed using specific antibodies against RXRα, PPARα, or PPARγ. Arrows indicate the supershifted complexes formed (that contain RXRα and PPARα or PPARγ). Incubation with an antibody against ETS transcription factors, used as negative control, did not result in any change in the pattern of bands. Competition experiments performed in Fig. 8D with a 100-fold molar excess of specific (UCP1-PPRE) or its mutated version (mutUCP1-PPRE, see legend of Fig. 8A) confirmed the presence of a nonspecific band (n.s.). Taken together, these findings demonstrate that both PPARα and PPARγ are present in brown fat cell nuclei and bind to UCP1-PPRE as heterodimers with RXRα.

The PPARα Ligand Wy 14,643 Induces ucp-1 Gene Expression in Brown Adipose Tissue in Vivo in Different Physiological Situations—To assess the in vivo significance of PPAR activators on the expression of the ucp-1 gene, mice at different physiological situations were injected with single doses of the PPARα-specific ligand Wy 14,643 or, for comparative purposes, of the PPARγ activator troglitazone. We have previously reported that sensitivity of gene expression to PPARα activators in acute treatments in vivo depends on the status of lipid metabolism able to provide endogenous PPARα ligands (36). In adult mice (Fig. 3), Wy 16,443 caused a moderate 1.5-fold increase in UCP-1 mRNA abundance in BAT. When lactating mice were analyzed, Wy 16,443 significantly increased (5-fold) UCP-1 mRNA levels. During lactation, functional atrophy of BAT, including diminished lipolytic and lipoprotein lipase activities, and reduced expression of the ucp-1 gene contribute to energy sparing (37, 38). In contrast, troglitazone only had a moderate effect on brown fat UCP-1 mRNA abundance.

When newborn mice at thermoneutrality were analyzed, injection of pups with Wy 14,643 caused a significant 3-fold rise in UCP-1 mRNA levels whereas injection of the PPARγ-ligand BRL 49653 did not significantly change UCP-1 mRNA expression. The action of PPAR agonists was specific for the ucp-1 gene since COII mRNA levels were essentially unaffected by PPAR activators in BAT (see Fig. 9, bottom). Present results demonstrate an acute regulation of the ucp-1 gene in vivo by the PPARα-ligand Wy 14,643 that is more potent than that observed for PPARγ ligands.

co-transfection of pRSV-RXRα was found (Fig. 5C), nearly 200-fold in the absence and 350-fold in the presence of 10 μM Wy 14,643. When the other PPAR subtypes were tested, co-transfection of PPARγ in the presence of RXRα caused a similar increase in (−4551)UCP1-CAT activity than that of PPARα, but no effect was observed due to PPARδ co-transfection (data not shown).

**CBP and PGC-1 Coactivate the PPARα-dependent Activation of the ucp-1 Gene Promoter—**We next analyze whether co-regulators CBP and/or PGC-1 were involved in mediating PPARα transcriptional regulation of (−4551)UCP1-CAT. Co-transfection of pCMX-CBP or pSV-PGC1 alone enhanced basal (−4551)UCP1-CAT activity 5- and 7-fold, respectively (Fig. 6). When co-transfected together with pSG5-PPARα, an additive effect was observed in the absence of PPARα ligand, but when 10 μM Wy 14,643 was added, a synergistic activation was detected (30-fold for CBP and nearly 40-fold for PGC-1). When both coregulators were co-transfected in the presence of PPARα, a further increase in (−4551)UCP1-CAT activity was observed. These results point to an involvement of both CBP and PGC-1 in coactivating PPARα and further increasing responsiveness of the ucp-1 gene promoter to PPARα-ligand.

**PPARα- and PPARγ-dependent Regulation Require the Same Element in the Upstream Region of the ucp-1 Gene Enhancer—**To determine the site in the 5′-region of the rat ucp-1 gene responsible for PPARα action, the effects of PPARα co-transfection on different deletion and double-point mutants of (−4551)UCP1-CAT were studied in transfected HepG2 and HIB-1B cells (Fig. 7). For comparative purposes, parallel co-transfection experiments were performed with PPARγ. Results in both cell lines and for each PPAR subtype, indicated that both PPAR subtypes share a responsive site located in the −2494/−2318 enhancer region of the ucp-1 gene. When a double-point mutation, in which the CC at positions −2472 and −2473 were changed to AA (see Fig. 8A), was introduced in both (−3628/−2283)UCP1-CAT and (−2494/−2318)UCP1-CAT vectors, responsiveness to both PPARα and PPARγ was abolished. Furthermore, when the −2494 to −2445 fragment
Fig. 5. PPARα-dependent induction of (−4551)UCP1-CAT expression in transiently transfected HIB-1B and HepG2 cells: influence of RXR cotransfection. A, HIB-1B cells were transfected with 1 µg of (−4551)UCP1-CAT vector, and included or not 0.5 µg of pSG5-PPARα, and/or 0.1 µg of pRSV-RXRα. After transfection, cells were exposed (dark bars) or not exposed (open bars) to 10 µM Wy 14,643 for 24 h. Results are shown as relative to the basal expression of (−4551)UCP1-CAT, which is set to 1. Bars are means of at least two independent experiments, each one done in duplicate. B, HepG2 cells were transfected with 1 µg of (−4551)UCP1-CAT vector together with increasing amounts of the expression vector pSG5-PPARα. After transfection, cells were exposed ( ) or not exposed (○) to 10 µM Wy 14,643 for 24 h. C, as in B, but 0.1 µg of pRSV-RXRα was also co-transfected. Points are means of at least two independent experiments, each one done in duplicate.

Fig. 6. Effects of CBP and/or PGC-1 co-transfection on the PPARα-dependent induction of (−4551)UCP1-CAT expression. HepG2 cells were co-transfected with 1 µg of (−4551)UCP1-CAT vector, and included or not 0.3 µg of pSG5-PPARα. When indicated, 0.1 µg of pCMX-CBP and/or pSV-PGC1 were also co-transfected. After transfection, cells were exposed (dark bars) or not exposed (open bars) to 10 µM Wy 14,643 for 24 h. Results are shown as relative to the basal expression of (−4551)UCP1-CAT, which is set to 1. Bars are means of at least two independent experiments, each one done in duplicate.

**DISCUSSION**

Here we have established that PPARα activators regulate the expression of the ucp-1 gene both in primary brown adipocytes and in BAT in vivo. Brown adipocytes differentiated in primary culture were used since they highly coexpress all PPAR subtypes, equally to BAT (24). In contrast, the HIB-1B brown adipocyte cell line lacks PPARα expression (24), and therefore, the results of previous studies using HIB-1B cells to determine the effects of PPAR activators on the expression of the ucp-1 gene must be viewed with caution. Present results also demonstrate that PPARα induces the rat ucp-1 gene promoter activity upon treatment with its specific ligand Wy 14,643, but it can also activate transcription in the absence of exogenously added ligand. This has been widely described for other PPARα-responsive gene promoters (32, 39), and could be explained by either the presence of endogenous activators, such as fatty acids or their metabolites, or by ligand-independent activity of these nuclear receptors (40). The responsiveness of the ucp-1 gene promoter to PPARα-ligand is increased by co-transfection with expression vectors for either coactivator CBP or PGC-1. Furthermore, the synergistic effect observed when adding both coactivators points to the involvement at the same time of CBP and PGC-1 in coactivating PPARα. In this way, PPARα can interact directly with CBP (41) and also with PGC-1 (42). In addition, CBP can form a complex with PGC-1 (43), thus providing multiple contact points to stabilize the complex assembly. Furthermore, CBP can also interact with other transcription factors, such as CREB and C/EBP, known to regulate transcription of the rat ucp-1 gene through its proximal regulatory region (22, 21).

By deletion and mutation analysis we have identified the PPARα-responsive element in the upstream enhancer region of the rat ucp-1 gene. This −2485/−2458 region contains a potential PPRE consensus formed by two direct repeats separated by one nucleotide (DR-1). Highly comparable elements are also found in the human and mouse ucp-1 genes (see Fig. 8A), indicating that these sequences may have an important regulatory role in response to PPARα. In fact, the murine element has been described to mediate PPARγ responsiveness (18). Our present results further demonstrate that the −2485/−2458 element in the rat ucp-1 gene behaves as a promiscuous responsive site to either PPARα and PPARγ activation, but not PPARδ. From the analysis of various natural PPREs, it has been reported that the binding strength and functional transactivation for each PPAR subtype on the same PPRE was similar (33). Only some significant PPARγ specificity was described, and it was related to the 5'-flanking sequence with respect to the DR-1 element, which is essential for PPARα binding (33). However, present results indicate a similar capacity of PPARα and PPARγ to bind and activate ucp-1 transactivation through the UCP1-PPRE. The predominant role of any subtype at any one time may thus depend on: 1) the relative amount of each subtype. For instance, PPARα and PPARγ gene expression in brown adipocytes are under opposite regulation by their ligands and retinoic acid: up-regulation of PPARα but down-regulation of PPARγ (24). 2) Cross-talk with other signaling pathways, like regulation of PPAR transactivation activity by MAP kinase-dependent phosphorylation, which enhances PPARα (44) but decreases PPARγ activity (45). 3) Ligand availability. Several PPAR ligands have been described to be highly subtype-specific (6), although identification...
of endogenous ligands and how their synthesis is regulated, is far from being established. 4) Interaction with coregulators. The interaction of PGC-1 with PPARα is ligand-dependent whereas that with PPARγ is not (42, 30). These and other possible events may determine which PPAR subtype activates transcription of ucp-1 in response to brown adipocyte physiological condition, mainly PPARγ in association with differentiation-dependent events or PPARα in coordination with increased lipid catabolism in active BAT.

Other PPAR target genes have been described to be induced by both PPARα and γ activators through the same PPRE (39, 46). However, since they have been studied in tissues such as liver, which highly expresses PPARα but not PPARγ, or WAT, which predominantly expresses PPARγ, tissue-specific regulation has been suggested. In contrast, BAT provides a model to study whether PPAR subtypes specifically regulate a PPRE in a target gene or whether a unique element behaves as a common site, as shown by our present findings in the ucp-1 gene promoter. For instance, the lipoprotein lipase (LPL) gene is up-regulated by PPARα (in liver) and PPARγ (in WAT) through the same PPRE (46). During BAT differentiation, induction of LPL allows for increased fatty acids delivery to brown adipocytes, which results in triglyceride accumulation, thus promoting the adipocyte phenotype. However, thermogenic stimulus

FIG. 7. Analysis of the PPARα- and PPARγ-dependent regulation of transiently transfected deletion or double-point mutants of the (−4551)UCP1-CAT. HepG2 and HIB-1B cells were transiently transfected with 1 μg of (−4551)UCP1-CAT or equivalent amounts of the deletion or double-point mutants illustrated on the left. Asterisks indicate the double-point mutated versions of (−3628/−2283)UCP1-CAT and (−2494/−218)UCP1-CAT, containing AA instead of CC at sites −2273 and −2272. Transfections included 0.1 μg of the expression vector pRSV-RXRα, and included or not, 0.3 μg of pSG5-PPARα (dark bars) or pSG5-PPARγ (open bars). Results are expressed as the -fold induction caused by PPAR co-transfection on each transfected construct. Bars are means of at least two independent experiments, each one done in duplicate.

FIG. 8. Electrophoretic mobility shift assays of the −2485/−2458 region of the rat ucp-1 gene. A, sequence corresponding to the −2485/−2458 region of the rat ucp-1 gene (UCP1-PPRE), was compared with a consensus PPRE (34) and to the analogous regions in the murine (−2499/−2472) and human (−3732/−3705) ucp-1 gene promoters (18, 35). Asterisks indicated the double-point mutant derivative version (mutUCP1-PPRE) in which the CC at positions −2273 and −2272 were changed to AA. The upper arrows show the putative alignments of three motifs closely related to an idealized half-site. B, gel mobility shift assay: the double-stranded oligonucleotide −2485/−2458 was end-labeled and incubated with 5 μl of in vitro transcribed/translated RXRα, alone or together with PPARα or PPARγ. Arrows indicate the corresponding heterodimers bound to the probe. Lane 1 showed that the mock lysate produced two nonspecific bands when incubated with the probe. C, super-shift assay: the labeled UCP1-PPRE probe was incubated with 5 μg of nuclear protein extract from differentiated primary brown adipocytes. When indicated, 1 μl of antiserum against RXRα, PPARα, PPARγ, or ETS (as negative control) were added. Arrows indicate the super-shifted bands. D, protein extracts from rat brown adipose tissue nuclei (5 μg) were incubated with the labeled UCP1-PPRE probe. Super-shift analysis was performed by incubation with 1 μl of antiserum against PPARα, PPARγ, or ETS. Oligonucleotide competitors, UCP1-PPRE (WT) and mutUCP1-PPRE (MUT), were added at a 100-fold molar excess relative to probe concentration. A nonspecific-binding band was detected (n.s.). Bracket indicates the specific-binding bands and arrows the super-shifted bands.
also up-regulates LPL to increase fatty acids uptake, which increases the supply of substrate for oxidation. Expression of LPL mRNA is increased by PPARα and γ activators in differentiated brown adipocytes, suggesting that LPL gene transcription in BAT could be activated by both PPARα and γ activators. Other genes, such as the fatty acid transport protein and the acyl-CoA synthetase genes, which also regulate cell uptake of fatty acids, might be similarly regulated in BAT since they are induced by PPARα and γ activators (39, 47).

Here we also demonstrate that in vivo activation of PPARα by Wy 14,643 up-regulates UCP-1 mRNA expression in BAT. The effects of the acute administration of this synthetic ligand are higher in those physiological situations (lactating dams and neonates between groups of treated mice and their respective vehicle treated female control which was set as 1. Statistical significance of comparison was set as 1. Statistical significance of comparisons between groups of treated mice and their respective vehicle treated controls are shown by: *, p < 0.05; **, p < 0.01. Comparison between Wy 14,643 and troglitazone treatment is shown by Δ, p < 0.05. B: representative Northern blot analysis of equal amounts of brown fat RNA (20 μg/lane) hybridized with the UCP-1 and COII probes, as described in the legend to Fig. 1.

In conclusion, PPARα directly regulates ucp-1 gene transcription and we propose that this transcriptional regulatory mechanism is a component of the coordinate control of thermogenic and lipid oxidation pathways in active BAT. Recently, PPARα has been implicated in obesity (54) and selective PPARα activators have been described to improve insulin sensitivity and reduce WAT mass (55). Part of these effects could be due to an increase in energy expenditure in BAT, and the positive action of PPARα on ucp-1 gene expression opens new perspectives on the molecular targets of PPARα involved in mediating these effects.

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