Rosiglitazone and Retinoic Acid Induce Uncoupling Protein-1 (UCP-1) in a p38 Mitogen-activated Protein Kinase-dependent Manner in Fetal Primary Brown Adipocytes*

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Brown adipose tissue expresses the thermogenic uncoupling protein-1 (UCP-1), which is positively regulated by peroxisome proliferator-activated receptor (PPAR) agonists and retinoids through the activation of the heterodimers PPAR/retinoid X receptor (RXR) and retinoic acid receptor (RAR)/RXR and binding to specific elements in the ucp-1 enhancer. In this study we show that in fetal rat brown adipocyte primary cultures the PPARγ agonist rosiglitazone (Rosi), as well as retinoic acids 9-cis-retinoic acid and all-trans-retinoic acid also have “extragenic” effects and induce p44/p42 and p38 mitogen-activated protein kinase (p38MAPK) activation. The latter is involved in UCP-1 gene expression, because inhibition of p38MAPK activation by PD169316 are mimicked by the antioxidant GSH, suggesting a role for reactive oxygenated species (ROS) generation in the increase of UCP-1 expression in response either to Rosi or 9-cis-retinoic acid. Thus, we propose that Rosi and retinoids act as PPAR/RXR and RAR/RXR agonists and also activate p38MAPK. These two coordinated actions could result in a high increase of transcriptional activity on the ucp-1 enhancer and hence on thermogenesis. PPARα and γ agonists but not retinoids also increase UCP-3 expression in fetal brown adipocytes. However, the regulation of UCP-3, which is not involved in thermogenesis, seems to differ from UCP-1 given the fact that is not affected by p38MAPK inhibition.

Brown adipose tissue (BAT) is the main site for non-shivering thermogenesis in small mammals. This function relies on the presence of the tissue-specific uncoupling protein-1 (UCP-1), which is located in the mitochondrial inner membrane and stimulates heat production by uncoupling oxidative phosphorylation from the respiratory chain (1). UCP-1 expression is under complex regulation. An enhancer element in the 5′-flanking region of the ucp-1 gene has been described with putative binding sites for the thyroid hormone receptor (THR), retinoic acid (RA) receptors (RAR and RXR), and the peroxisome proliferator-activated receptor (PPAR) (2, 3). Although β-adrenergic stimulation is the main physiological pathway that induces thermogenesis and UCP-1 expression in BAT, it is likely to be mediated by the induction of the PPARγ coactivator (PGC-1), a master coactivator that binds PPARγ, PPARα, THR, RAR, and RXR (4, 5). BAT also expresses the recently described UCP-2 and UCP-3, the latter being restricted to two thermogenic tissues (BAT and skeletal muscle). UCP-3 has shown to uncouple respiration when expressed ectopically in yeast and mammalian cells (6, 7), and a role for UCP-3 in the regulation of energy expenditure has been reported (8) as a response to nutritional states, with a key role for PPARα and PPARγ ligands as positive regulators of UCP-3 gene expression (9, 10).

We have previously reported (11, 12) that the PPARγ agonist, rosiglitazone (Rosi), increased UCP-1 and UCP-3 mRNA levels in fetal rat brown adipocytes prior to the acquisition of the catecholaminergic neuronal input. In contrast, the PPARα ligand Wy14643 induced UCP-3 but not UCP-1 expression, although it was able to transactivate the −455UCP-1-chloramphenical acetyltransferase (CAT) reporter gene (11, 12). The effect of Rosi on UCP-1 mRNA levels is mediated by increasing the transcription of the gene through the binding of the PPAR/RXR heterodimer to the PPAR response element (PPRE) described in the enhancer (2). PPREs have also been described in the 5′-flanking region of the human ucp-3 gene (13, 14). Among the coactivators that bind to PPAR, PGC-1 is highly expressed in BAT and plays a key role in adaptive thermogenesis. Because recent reports have described modulation either of PPARα, PPARγ, and PGC-1 activity by phosphorylation via p44/p42 mitogen-activated protein kinase (MAPK) (15, 16), p38MAPK (17, 18), and/or protein kinase A (PKA) (19), we decided to explore the involvement of those kinases in the UCP-1 and UCP-3 induction by PPARα and α agonists. This paper describes p38MAPK and p44/p42MAPK activation triggered either by Rosi or 9-cis-RA or all-trans-RA treatment in fetal rat brown adipocytes, with p38MAPK implication in UCP-1 but not UCP-3 expression.

EXPERIMENTAL PROCEDURES

Materials—Wy14643 was from Biomol Research Laboratories (Plymouth, UK). Rosiglitazone was kindly provided by Dr. S. A. Smith (GlaxoSmithKline, Harlow, UK). PD169316 and PD98059 were purchased from Calbiochem-Novabiochem. Myelin basic protein (MBP), BSA, Bt2cAMP, 9-cis-RA and all-trans-RA were from Sigma. Fetal calf

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2 The abbreviations used are: BAT, brown adipose tissue; BSA, bovine serum albumin; CAT, chloramphenical acetyltransferase; ERR, extracellular signal-regulated kinase; FAS, fatty acid synthase; FCS, fetal calf serum; MAPK, mitogen-activated protein kinase; MBP, myelin basic protein; MEM, minimal essential medium; PBS, phosphate-buffered saline; PD, PD98059; PDc, PD169316; PGC-1, PPARγ coactivator; PPRE, PPAR response element; RA, retinoic acid; RAR, RXR receptor; ROS, reactive oxygenated species; Rosi, rosiglitazone; RXR, retinoid X receptor; UCP, uncoupling protein.
serum (FCS), phosphate buffered saline (PBS), culture media, and Triozol were from Invitrogen. Nylon membranes were GeneScreen ™ (PerkinElmer Life Sciences). Autoradiographic films were Kodak X-O-MAT/AR (Eastman Kodak). [α-32P]-dCTP, [γ-32P]-ATP, [3H]-chloramphenicol, and the multiprimer DNA-labeling system kit were purchased from Amersham Biosciences. All other reagents were used of the purest grade available. The cDNAs used as probes were UCP-1, UCP-3, and UCP-4 constructs used for transfection experiments were kindly provided by D. Ricquier (Meudon, France) (22) and pCMV-β-galactosidase supplied by Stratagene. The anti-phospho- and anti-(p44/p42MAPK and p38MAPK) antibodies were from Cell Signaling (Beverly, MA). PPARγ antibody “sc-1984” was from Santa Cruz Biotechnology.

Cell Culture—Fetal brown adipocytes were obtained from interscapular brown adipose tissue of 20-day-old Wistar rat fetuses and isolated by collagenase dispersion as described (23). Isolated cells were plated at 1.5 × 10⁶ cells/60-mm tissue culture dishes in 2.5 ml of minimal essential medium (MEM) with Earle’s salts supplemented with 10% FCS, the final medium (MEM) with Earle’s salts supplemented with 10% FCS, and then fed with serum-free BSA-MEM medium (either in the absence or presence of Rosi (0.1 mM) for 24 h. The cells were then harvested, and the lysates were prepared for CAT and β-galactosidase activity assays. CAT activity was determined by incubating 50 μl of cell extracts with 0.25 μCi of [14C]-chloramphenicol and 0.5 mM acetyl CoA in 20 μl of assay buffer for 2 h. The amount of acetylated substrate was directly quantified with a radioimaging device (Fujiﬁlm BAS-1000).

RNA Extraction and Analysis—For Northern-blot analysis of RNA, at the end of the culture time the cells were washed twice with ice-cold PBS and lysed directly with Triozol following the protocol supplied by the manufacturer for total RNA isolation (11). Total cellular RNA (15 μg) was submitted to Northern blot analysis, i.e. electrophoresed on 0.9% agarose gels containing 0.66 μl formaldehyde, transferred to GeneScreen ™ membranes using a VacuGene blotting apparatus (LKB-Pharmacia) and cross-linked to the membranes by UV light. Hybridization was in 0.25 mM NaHPO4, pH 7.2, 0.25 M NaCl, 100 μg/ml denatured salmon sperm DNA, 7% SDS, and 50% deionized formamide containing denatured 32P-labeled cDNA (10⁶ cpm/ml) for 24 h at 42 °C as described (24). Complementary DNA labeling was carried out with [α-32P]-dCTP to a speciﬁc activity of 10⁶ cpm/μg of DNA by using multiprimer DNA-labeling system kit. For serial hybridization with different probes, the blots were stripped and subsequently rehybridized as needed in each case. Membranes were subjected to autoradiography, and relative densities of the hybridization signals were determined by densitometric scanning of the autoradiograms in a laser densitometer (Amersham Biosciences).

Transient Expression—The plasmid constructs used for transfection were −4551UCP-1-CAT, where the CAT reporter gene is under the control of a 4551 full-length 5′-flanking region of rat UCP-1, and pCMV-β-galactosidase, a viral promoter-driven expression of the reporter gene β-galactosidase. Fetal primary brown adipocytes were cultured for 24 h in the presence of 10% FCS-MEM and then transiently transfected according to the calcium phosphate-mediated protocol with 10 μg of DNA-CAT together with 2 μg of DNA-β-gal (to monitor transfection efﬁciency) as described previously (25). After 4 h of incubation, cells were washed with 3 ml of 15% PBS-glycerol for 2 min, washed, and then fed with serum-free BSA-MEM medium (either in the absence or presence of Rosi (0.1 mM) for 24 h. The cells were then harvested, and the lysates were prepared for CAT and β-galactosidase activity assays. CAT activity was determined by incubating 50 μl of cell extracts with 0.25 μCi of [14C]-chloramphenicol and 0.5 mM acetyl Coenzyme A in 0.25 mM Tris (pH 7.8) at 37 °C for 12 h, and samples were then submitted to thin layer chromatography. The amount of acetylated substrate was directly quantiﬁed with a radioimaging device (Fujifilm BAS-1000).

CAT enzyme activity was expressed as a percentage of acetylated [14C]-chloramphenicol normalized to the internal control, β-galactosidase assayed according to the Stratagene protocol. Routinely, we performed direct β-galactosidase staining on parallel dishes, and observation under inverse light microscopy demonstrated that blue cells were mainly brown adipocytes with their characteristic multilocular fat droplets phenotype.

Western Blotting—Cells were lysed in the lysis buffer (25 mM Hapes, 0.3 mM NaCl, 20 mM glycerophosphate, 0.2 mM EDTA, 0.1% Triton X-100, 0.5 mM dihydrothreitol, 0.1 mM orthovanadate, 1 mM phenylmethanesulfon- nyl fluoride, 2 μg/ml leupeptin, pH 7.5), and the cellular proteins (30 μg) were submitted to SDS-PAGE, transferred to Immobilon membranes, and blocked using 5% nonfat dried milk in 10 mM Tris-HCl and 150 mM NaCl, pH 7.5, and incubated overnight with several antibodies as indicated in each case in 0.05% Tween 20, 1% nonfat dried milk in 10 mM Tris-HCl, and 150 mM NaCl, pH 7.5. Immunoreactive bands were visualized using the enhanced chemiluminescence (ECL-Plus) Western blotting protocol (Amersham Biosciences).

p38MAPK Activity Assay—Cells were extracted with lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 2 mM EDTA, 1 mM EGTA, 1 mM phenylmethanesulfonfyl fluoride, 25 μg/ml leupeptin, and 25
RESULTS

Fetal rat brown adipocytes, when isolated and allowed to attach to the plastic dishes for 4 h in 10% FCS-MEM, express adipogenic (FAS) and thermogenic (UCP-1) markers. After 20 h of culture in the absence of serum, UCP-1 mRNA levels decrease dramatically, facilitating the study of its up-regulation by several hormones/signals. Under these conditions, treatment for 24 h with noradrenaline or triiodothyronine or 9-cis-RA or insulin-like growth factor-1 is able to induce UCP-1 gene expression (27) (25). We have previously used the same culture conditions to demonstrate that Rosi per se and in the absence of any other exogenously added signal increases UCP-1 and UCP-3 mRNA levels in a time- and dose-dependent manner and induces activation of the full promoter UCP-1-CAT (11) (12). To explore the possible involvement of the extracellular signal regulated kinases (ERKs), p38MAPK, and/or PKA on UCP-1 induction by Rosi, we used chemical inhibitors for those kinases as follows: PD98059 (PD), 20 μM, to inhibit ERKs; PD169316 (PD*) , 800 nM, as a p38MAPK inhibitor; and H89, 10 μM, to inhibit PKA activity. The efficiency of PD* and PD to inhibit p38MAPK activity and p44/p42MAPK phosphorylation at the doses used was tested in Fig. 3, B and C. Furthermore, H89 at 10 μM completely blocked UCP-1 mRNA induction as well as FAS mRNA repression by Bt2cAMP (Fig. 1 B). Fetal rat brown adipocytes were treated for 24 h with or without 10 μM Rosi either in the absence or presence of the inhibitors. At the end of the culture period, total RNA was extracted and analyzed for UCP-1 expression by Northern blot (Fig. 1 A). As expected, Rosi increased UCP-1 mRNA levels by 4-fold, and this effect was unmodified in the presence of PD or H89. However, the inhibition of p38MAPK activity with PD* resulted in an almost complete blockade of UCP-1 induction by Rosi without a significant effect on the basal UCP-1 mRNA levels found in untreated cells. To validate whether the increase in UCP-1 expression produced by Rosi in a p38MAPK-dependent manner was independent of differentiation-inducing properties of Rosi or p38MAPK (28), the expression of the adipogenic marker FAS was also analyzed (Fig. 1A). Rosi treatment for 24 h did not induce FAS mRNA levels and did not increase intracellular lipid content (data not shown), indicating that the effects of Rosi are related to specific thermogenic gene expression rather than to a differentiation program. Furthermore, no changes on
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Fig. 3. Rosi induces p38MAPK and p44/p42MAPK activation in brown adipocytes. A and C, after 20 h of serum deprivation, fetal brown adipocytes were cultured in the absence or presence of 10 μM Rosi for 1, 3, and 6 h. A treatment for 6 h with Rosi plus PD was also performed as shown in C. Total protein (30 μg) was submitted to SDS-PAGE, blotted onto nylon membrane, immuno-detected with anti-phospho-p38MAPK, anti-p38MAPK, anti-phospho-p44/p42MAPK, or anti-p44/p42MAPK and developed with ECL chemiluminescence. Representative autoradiograms (left panels) and densitometric analysis expressed as arbitrary units (means ± S.E. (n = 6) (right panels) are shown. Statistical significance was tested as described under “Experimental Procedures,” and differences between values in the presence of Rosi versus control are represented by *. For *, p < 0.01. B, serum-deprived cells were treated for 3 h in the absence (C) or presence of 10 μM Rosi ± 800 nM PD, lysed, and immunoprecipitated with anti-p38MAPK antibody. The resulting immune complexes were assayed for MBP phosphorylation. A representative experiment is shown (left panel) as well as the densitometric analysis (right panel) for p38MAPK activity. Results are phosphorylated MBP levels in arbitrary units and are the means ± S.E. (n = 8). Statistical significance was tested as described under “Experimental Procedures,” and the differences between values in the presence of Rosi versus control are represented by *, and the differences between values in the presence of Rosi plus PD versus Rosi are represented by ∆. For * and ∆, p < 0.01.

FAS mRNA expression were observed regardless of the presence or absence of the inhibitors (Fig. 1A).

We also performed transient transfections with −4551UCP-1-CAT (where the CAT reporter gene is under the control of a 4551-bp full-length 5′-flanking region of the rat UCP-1 promoter) to test whether the inhibition was produced on the transcription process. Fetal primary brown adipocytes were transiently co-transfected with 10 μg of −4551UCP-1-CAT together with 2 μg of pCMVβ-galactosidase for internal control of transfection. Upon transfection, cells were cultured for 24 h in a serum-free BSA-MEM medium in the absence or presence of Rosi with or without PD169316. At the end of the culture period, cells were collected and assayed for CAT activity.

As depicted in Fig. 2A, Rosi increased −4551UCP-1-CAT activity 3-fold, and this stimulation was totally precluded when the p38MAPK inhibitor was present in the culture medium. These results indicate that p38MAPK but not p44/p42MAPK or PKA activity is necessary for Rosi-induced UCP-1 gene transcription. To check whether the inhibition of p38MAPK was affecting the levels of the PPARγ protein, a direct Western blot was performed (Fig. 2B). Similar PPARγ protein content was detected in brown adipocytes either in the absence or presence of PD. Furthermore, we checked whether nuclear protein binding to the PPRE of the UCP1 enhancer was affected by the inhibition of p38MAPK, either in the presence or absence of Rosi. Nuclear extracts from cells cultured for 24 h with or without Rosi and/or PD were used in gel mobility shift assays using UCP-1-PPRE double-stranded oligonucleotide as a probe (Fig. 2C). Protein binding to DNA was observed in control brown adipocytes, and this binding was essentially unmodified either by the presence of Rosi or PD. This band seems to be specific, because it disappears in competition experiments with a 100-fold molar excess of unlabeled PPRE probe.

Many ligands for nuclear receptors act not only on gene expression activating nuclear receptors, but also affect cytosolic signaling pathways (29–31). To check the possibility that Rosi could be inducing p38MAPK activation, we treated brown adipocytes with Rosi for different period of time, and cell lysates were analyzed for dual phosphorylation (Thr-180/Tyr-182) of p38MAPK by Western blot with a polyclonal antibody. No phosphorylation of p38MAPK was detected in control cells cultured for 20 h in a serum-free BSA-MEM medium. However, Rosi treatment for 1 h induced p38MAPK phosphorylation, which was maintained up to 6 h (Fig. 3A). The changes observed in the amount of phospho-p38MAPK reflect changes in the activity, because the protein levels of p38MAPK are similar in all the conditions. Furthermore we performed p38MAPK activity assay with protein lysates from control cells and cells treated with Rosi for 3 h and obtained similar results. Rosi increased MBP phosphorylation 3-fold in anti-p38MAPK immunoprecipitates, and this activation was prohibited in cells cotreated with Rosi and PD, validating the use of this chemical compound as a p38MAPK inhibitor (Fig. 3B). Western blot analysis with anti-phospho-p44/p42MAPK antibody revealed...
that the presence of Rosi for 3 h also increases p44/p42MAPK activity, which is further elevated after 6 h (Fig. 3C). This p44/p42MAPK activation is inhibited by PD, validating the use of this chemical compound in brown adipocytes. 0.1% Me2SO, the vehicle used, did not produce any effect (data not shown). These data clearly show that Rosi, besides being a PPARγ agonist, stimulates kinase activities that can modulate its effects on gene expression.

Although p38MAPK activation, but not p44/p42MAPK, is involved in UCP-1 gene induction by Rosi, this kinase does not play any role in UCP-3 expression either induced by Rosi or by the PPARα agonist Wy14643 as is assumed from the results obtained by Northern-blot analysis and represented in Fig. 4, where it is shown that UCP-3 expression is induced in cells treated with Rosi or Wy14643 regardless of the presence of the p38MAPK inhibitor PD169316.

Because PPARs bind to DNA as heterodimers taking RXR as partner, and RXR ligands also increase UCP-1 gene transcription, we wanted to check the possibility that p38MAPK activation was also involved in UCP-1 induction by RXR ligands. Brown adipocytes were cultured for 24 h with 9-cis-RA (agonist for RXR and RAR) or all-trans-RA (RAR agonist) in the absence or presence of the p38MAPK inhibitor (PD169316). Northern-blot analysis for UCP-1 expression revealed that 9-cis-RA treatment produced a huge increase in UCP-1 mRNA levels (12-fold) that was inhibited 50% in the presence of PD169316. The RAR agonist also induced UCP-1 expression but to a lesser extent than 9-cis-RA, this effect also being precluded by inhibition of p38MAPK. As previously published (12), none of the retinoids had any effect on UCP-3 expression (Fig. 4). Because p38MAPK activation also seemed to be involved in UCP-1 induction by retinoic acids, we decided to examine whether 9-cis-RA and all-trans-RA also activated p38MAPK; this was monitored by a Western blot of phosphorylated p38MAPK. As shown in Fig. 5, 9-cis-RA induced p38MAPK phosphorylation with a timing pattern similar to Rosi. p44/p42MAPK activation was also observed upon 9-cis-RA stimulation. Regarding all-trans-RA, it also activated p44/p42MAPK and p38MAPK but less strongly than 9-cis-RA.

A very recent paper, which showed that MAPK activation by the PPARγ agonists 15-deoxy-Δ12,14-prostaglandin J2 (dPGJ2) and ciglitazone is produced through a mechanism involving reactive oxygenated species (ROS) (32), prompted us to examine whether ROS generation could be involved in UCP-1 induction by Rosi and/or retinoic acids. Brown adipocytes were or were not pretreated 30 min with an antioxidant agent, reduced GSH, before the addition of Rosi or 9-cis-RA and further cultured for 24 h. UCP-1 expression was analyzed by Northern-blot, and the results depicted in Fig. 6 show that the presence of GSH inhibited the increase in UCP-1 mRNA levels elicited by either Rosi or 9-cis-RA.

**DISCUSSION**

Nuclear PPARs activate the transcription of multiple genes involved in lipid metabolism as well as the thermogenic protein UCP-1 and the recently described UCP-2 and UCP-3. PPARs are activated by ligand binding, which induces conformational changes leading to the recruitment of several coactivators (33). Furthermore, some agonists for PPARs, including the prostaglandin 15d-PGJ2 and the thiazolidinediones ciglitazone, pioglitazone, and troglitazone, have shown PPAR-independent effects, activating different cytosolic signaling pathways (ERK or c-Jun NH2-terminal kinase or p38MAPK) depending on the cellular system (30, 32, 34). It has also been reported that retinoic acids induce ERKs and p38MAPK activation in some cellular systems (29, 31, 35). To our knowledge, the results presented in this study describe for the first time p44/p42 and p38MAPK activation by 9-cis-RA and all-trans-RA as well as Rosi in fetal rat brown adipocytes. Kinetics for both activations are different. Meanwhile p38MAPK activation is rapid and sustained, whereas p44/p42MAPK phosphorylation delays and happens gradually. The biological effects of p44/p42MAPK activation are unknown. Phosphorylation by p44/p42MAPK can modulate PPAR activity, both in a positive and negative man-
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**Fig. 5.** 9-cis-RA and all-trans-RA induce p38MAPK and p44/p42MAPK phosphorylation. Fetal brown adipocytes, after 20 h of serum deprivation, were cultured in the presence or absence of 400 nM 9-cis-RA or 400 nM all-trans-RA for 1, 3, and 6 h. Total protein (30 μg) was submitted to SDS/PAGE, blotted onto nylon membrane, immunodetected with anti-phospho-p38MAPK (P-p38MAPK) or anti-p38MAPK (P-p38MAPK) or anti-phospho-p44/p42MAPK (P-p44MAPK, P-p42MAPK) or anti-p44/p42MAPK (B) and developed with ECL chemiluminescence. Representative autoradiograms (left panels) and densitometric analysis expressed as arbitrary units (means ± S.E. n = 6) (right panels) are shown. Statistical significance was tested as described under "Experimental Procedures," and the differences between values in the presence of 9-cis-RA or all-trans-RA versus control are represented by *. For *, p < 0.01.

**Fig. 6.** GSH impairs Rosi and 9-cis-RA induction of UCP-1. Fetal brown adipocytes, after 20 h of serum deprivation, were pre-treated or not with 0.5 mM reduced GSH and further cultured for 24 h with 10 μM Rosi or 400 nM 9-cis-RA. Total RNA was extracted from the different conditions, and 15 μg of it was submitted to Northern blot analysis and hybridized with labeled UCP-1 cDNA. A final hybridization with the 18 S rRNA cDNA was performed for normalization. A representative autoradiogram and a densitometric analysis of the UCP-1 mRNA level after standardization using the 18 S rRNA signal are shown. Results (arbitrary densitometric units) are means ± S.E. (n = 6). Statistical significance was tested as described under "Experimental Procedures;" and the differences between values in the presence of Rosi or 9-cis-RA versus control are represented by *, and the differences between values in the presence of Rosi or 9-cis-RA plus GSH versus the same treatment without GSH are represented by Δ. For * and Δ, p < 0.01.

p38MAPK phosphorylation by Rosi fits tightly to the time frame for UCP-1 gene induction (11), and inhibition of p38MAPK activity prevents UCP-1 mRNA increase by this thiazolidinedione or retinoids. This effect seems to be specific for the thermogenic protein UCP-1 and not related to the differentiation-inducing properties of Rosi or p38MAPK, because the expression of the adipogenic gene FAS remains unmodified by either the presence of Rosi or PD* in fetal rat brown adipocytes.

The inhibition of rosiglitazone-induced UCP-1 by PD* is not mediated by changes either in PPARα levels or in nuclear protein binding to UCP-1-PPRE. However, we cannot exclude the possibility that PD* could be blocking the binding of Rosi to PPARα, although it does not seem likely because other effects of Rosi (such as UCP-3 induction) are not modified by the presence of PD*. PPAR, RXR, and RAR bind PGC-1, a master coactivator involved in thermogenesis and UCP-1 expression. PGC-1 has been shown to be susceptible to phosphorylation by p38MAPK, resulting in a positive regulation of its transcriptional activity (18, 37). Therefore, it is tempting to speculate that Rosi and retinoids would act by two convergent mechanisms: 1) binding to specific nuclear receptors; and 2) switching on signaling pathways (p38MAPK) that positively modulate coactivators involved in activating transcription of specific genes such as UCP-1. In contrast to our results, Oberkofler et al. (38) have reported that the inhibition of p38MAPK signaling decreases the stimulatory effects of PPARα agonists treatment on UCP-1 transcription without reducing the response to thia-
zolidinediones or retinoid acids. This study was performed in the human brown adipocyte cell line PAZ6 co-transfected with expression vectors for h-PPC-1 and UCP-1-reported plasmids, whereas our experimental conditions are closer to a physiological situation because the primary culture is used with no exogenously expressed protein. These differences or even cell type (human versus rat) could explain the contrasting results. In any case, both studies show that p38MAPK activity regulates PPAR/RXR action on UCP-1 gene transcription.

Regarding UCP-3, we show that both Rosi and Wy14643 (PPARγ and PPARα agonists respectively) induce UCP-3 expression, but the mechanism seems to differ from UCP-1 because p38MAPK inhibition does not affect the response. This could imply that there is no participation of PGC-1 in UCP-3 gene expression as has been reported in white adipose or skeletal muscle cells ectopically expressing PGC-1 (4, 39) or even that the PPRE found in the ucp-3 promoter is not functional and that other response elements are responsible for Rosi- or Wy14643-induced UCP-3 gene expression as was reported for UCP-2 (40). In conclusion, Rosi or retinoid treatment activates p44/p42MAPK and p38MAPK, the latter being involved in UCP-1 but not in UCP-3 gene expression in fetal rat brown adipocytes.

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