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Evidence for a Common Mechanism of Action for Fatty Acids and Thiazolidinedione Antidiabetic Agents on Gene Expression in Preadipose Cells

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SUMMARY

In diabetic rodents, thiazolidinediones are able to improve insulin sensitivity of target tissues and to reverse, at least partially, the diabetic state. The effects of these drugs on phenotypic expression in various tissues, including adipose tissue, have been reported. We report here that a new thiazolidinedione compound, BRL 49653, exerts, in preadipose cells, potent effects on the expression of genes encoding proteins involved in fatty acid metabolism. These effects of BRL 49653 in Ob 1771 preadipose cells are similar, in terms of kinetics, reversibility, specificity of genes affected, and requirement for protein synthesis, to those already described for natural or nonmetabolizable fatty acids. Moreover, when used at submaximally effective concentrations, BRL49653 and 2-bromopalmitate act in an additive manner to induce gene expression in preadipose cells, but this additivity of effects is lost when one of the compounds is used at a maximally effective concentration. These observations, suggesting similar mechanisms of action for thiazolidinediones and fatty acids, are strongly supported by the demonstration that (i) both molecules activate, in a heterogolous *trans*-activation assay, the same nuclear receptor of the steroid/thyroid hormone nuclear receptor superfamily and (ii) transfection of 3T3-C2 fibroblasts with an expression vector for this nuclear receptor confers thiazolidinedione inducibility of adipocyte lipid-binding protein gene expression.

Thiazolidinediones are a new class of antidiabetic agents that improve insulin sensitivity in rodent models of non-insulin-dependent diabete mellitus, such as *ob/ob* and *db/db* mice and Zucker fatty rats. This is associated with decreases in glucose, triglycerides, and insulin (1). Because the effects of thiazolidinediones are most marked in obese animals, it has been postulated that adipose tissue must play a central role in their pharmacological action. In adipocytes from *ob/ob* mice treated with ciglitazone, the basal and insulin-stimulated rates of glucose metabolism and lipogenesis are increased (2, 3). Pioglitazone administration to obese KKA^y mice corrects the deficit in glucose transport by up-regulating the expression of insulin-regulatable glucose transporter type 4 in adipose tissue (4). *In vitro*, thiazolidinediones act as potent activators of cell differentiation in 3T3-L1 cells. Pioglitazone, in combination with insulin or insulin-like growth factor-1, dramatically increases adipocyte differentiation (5), whereas in ST13 preadipose cells the ciglitazone metabolite ADD 4743 exerts its potent adipogenic effects even in the absence of insulin (6).

The mechanisms by which thiazolidinediones exert their various effects are not well understood. It has been demonstrated that pioglitazone is able to regulate ALBP gene expression in 3T3-L1 cells (7). We have reported that fatty acids are involved in the regulation of expression of adipose-related genes, such as ALBP and ACS, in Ob 1771 preadipose cells (8, 9). Short term exposure of preadipose cells, i.e., committed, non-terminally differentiated cells that express early markers and in which genes such as ALBP or ACS are transcriptionally inactive, to long chain fatty acids led to induction of these genes and to an accumulation of the corresponding mRNAs. This effect of fatty acids was dose dependent and reversible upon removal of the inducer. A nonmetabolizable fatty acid, 2-bromopalmitate, was shown to be a better inducer than palmitate, demonstrating that fatty acid metabolism was not required for the induction of gene expression in preadipose cells (10).

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ABBREVIATIONS: ALBP, adipocyte lipid-binding protein (also called aP2, p422, and aFABP); ACS, acyl-CoA synthase; BRL 49653, 5-(4-[(*N*-methyl-*N*-(2-pyridyl)amino)ethoxy]benzyl)thiazolidine-2,4-dione; CAT, chloramphenicol acetyltransferase; FAAR, fatty acid-activated receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPDH, glycerophosphate dehydrogenase; hGR, human glucocorticoid receptor; PPAR, peroxisomal proliferator-activated receptor; SVF, stromal vascular fraction; MMTV, mouse mammary tumor virus; MAL1, keratinocyte lipid-binding protein; ARE, adipocyte regulatory element.

It is now well established that transcriptional effects of fatty acids are mediated through activation of nuclear receptors of the steroid/thyroid hormone receptor superfamily. Such receptors, called PPARs, have been identified in various mammalian tissues and are activated by a broad spectrum of amphipathic carboxylates such as fatty acids and fibrates (11–14). Recently, we have isolated from mouse preadipocytes a cDNA encoding a new member of this steroid/thyroid hormone receptor superfamily that is likely to be involved in the transcriptional effects of fatty acids in adipose tissue. Based upon these properties, it has been termed FAAR (15). Because thiazolidinediones can also be considered as amphipathic molecules, it could be hypothesized that these compounds exert their effects on gene expression in preadipose cells by a mechanism similar to that mediating the fatty acid effects. To test this hypothesis, we investigated the effects of a new compound of the thiazolidinedione class (16), BRL 49653, on the control of gene expression in cells of the preadipose Ob 1771 clonal line. From these studies it can be concluded that BRL 49653 and 2-bromopalmitate increase the transcription of the same set of genes, and the two compounds act in an identical manner to activate the ALBP gene. Furthermore, BRL 49653 is able to activate the FAAR, which is expressed in preadipose cells. These observations strongly suggest that thiazolidinediones and fatty acids activate gene expression in preadipose cells by similar mechanisms.

Materials and Methods

Cell culture. Ob 1771 cells (17) were plated at a density of 2×10^3/cm^2 and were grown in Dulbecco's modified Eagle's medium supplemented with 8% bovine serum, 200 units/ml penicillin, 50 μg/ml streptomycin, 33 μM biotin, and 17 μM pantothenate. This medium is termed standard medium. Media were changed every other day and confluence was reached within 5 days. Thiazolidinediones and 2-bromopalmitate were dissolved at a concentration of 50 mM in dimethylsulfoxide and ethanol, respectively. Aliquots were added immediately to standard medium to obtain the final concentration as indicated. This medium was prewarmed at 37° for 15 min and then added to the cells. Control experiments were performed to exclude any effects of ethanol or dimethylsulfoxide. Where indicated, BRL 49653 and 2-bromopalmitate were removed from the culture medium by washing of the cells with standard medium at 37° (two washes of 15 min each).

Stable transfectants. An expression vector (pSG5/FAAR) was obtained by insertion of the coding sequence (nucleotides 1–1440) of FAAR into the *Bam*HI site of the pSG5 vector (Stratagene). 3T3-C2 cells (18) (2×10^5/100-mm plate) maintained in standard medium were co-transfected with 1 μg of pMAMneo (Clontech) and 20 μg of pSG5 without (control cells) or with (FAAR cells) the FAAR insert, using the DOTAP (*N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-triethylammonium methylsulfate) transfection method. Geneticin was added to the medium 48 hr later and geneticin-resistant clones were selected and tested for responses to BRL 49653.

Adipose tissue culture. Epididymal fat pads from 8-week-old Wistar rats were rinsed in phosphate-buffered saline, pH 7.4, chopped into approximately 150-mm^3 pieces, and incubated in Dulbecco's modified Eagle's medium supplemented with 5 μg/ml insulin, 10 μg/ml tranferrin, 0.2 nM triiodothyronin, 20 nM sodium selenite, and 100 μM sodium ascorbate, in the absence or presence of 0.8 μM BRL 49653. Twenty hours later, adipose tissue fragments were digested with collagenase and cells of the SVF were isolated by centrifugation, as described previously (19).

RNA analysis. RNA samples were prepared as described by Chomczynski and Sacchi (20). RNA was analyzed as described previously (21). Results were quantitated by densitometry using an LKB Ultroscan KL laser densitometer. All measurements were taken from within the linear response of the integrated peaks as a function of immobilized RNA. GAPDH mRNA signals were used as an internal standard.

Hybrid receptor activation assay. The hybrid receptor hGR/FAAR was prepared by PCR mutagenesis (15). This construction encoded a chimeric protein corresponding to the A/B and C domains of hGR (amino acids 1–486) and the D and E/F domains of FAAR (amino acids 137–440). The chimeric construct was verified by sequencing and transfected into COS-1 cells with the plasmid MMTV-CAT, in which the expression of CAT is under the control of the glucocorticoid-responsive MMTV promoter. Transient transfection was performed in 60-mm dishes with cells maintained in phenol red-free medium supplemented with 8% activated charcoal-treated fetal calf serum, using 5 μg of both plasmids and the DOTAP transfection reagent (Boehringer Mannheim, Meylon, France). After 8 hr, cells were washed and incubated in the same medium in the presence of various ligands. Cell extracts were prepared 48 hr after transfection and assayed for CAT enzyme activity, using the CAT enzyme assay system (Promega, Charbonnieres, France). Control experiments were performed with cells transfected either with the MMTV-CAT plasmid alone or with the hGR expression plasmid and the reporter gene. Transfection experiments were performed in triplicate.

Materials. Culture media were obtained from GIBCO (Cergy-Pontoise, France). 2-Bromopalmitate was from Aldrich Chimie (Saint-Quentin, France). Bovine serum and other chemical products were purchased from Sigma Chimie (Saint-Quentin, France). Radioactive materials, the random priming kit, Hybond membranes, and Hyperfilm MP were from Amersham (Les Ullis, France).

Results

Effects of BRL 49653 on gene expression in preadipose Ob 1771 cells. The effects of exposure of 1-day postconfluent Ob 1771 cells to either BRL 49653 (100 μM) or 2-bromopalmitate (100 μM) on the level of expression of several genes were investigated by Northern blot analysis. As shown in Fig. 1, cells were already committed to differentiate at this time, because they were expressing the early marker pOb24/A2COL6 (22). However, they were not yet terminally differentiated, as demonstrated by the absence of late markers such as ALBP, ACS, and GPDH mRNAs (Fig. 1, *lanes 1*). Exposure to BRL 49653 for 24 hr led to emergence of ALBP and ACS mRNAs, whereas the level of pOb24/A2COL6 mRNA remained unchanged (Fig. 1, *lane 3*). MAL1 mRNA, which encodes a newly described fatty acid-binding protein present in various tissues including adipose tissue (23), showed a different pattern of expression. This mRNA was already expressed at a low, but significant, level in 1-day postconfluent cells (Fig. 1, *lane 1*), and its expression was enhanced in cells exposed to BRL 49653 (Fig. 1, *lane 3*). In contrast to the aforementioned adipose-related mRNAs, GPDH mRNA remained undetectable at this time. Additional experiments, not presented here, have shown that other adipose-related mRNAs, such as adipsin, hormone-sensitive lipase, and insulin-regulatable glucose transporter type 4 mRNA remained undetectable even in cells treated for 24 hr with BRL 49653. A similar pattern of expression was apparent when 2-bromopalmitate was used instead of BRL 49653 (Fig. 1, *lanes 2* versus *lanes 3*), indicating that both compounds exert positive effects on the expression of the same set of genes, i.e., ALBP, ACS, and MAL1. Similarly, the expression of the pOb24/A2COL6 gene was not modified, whereas no activation of GPDH or other terminal differentiation-related genes could be observed.

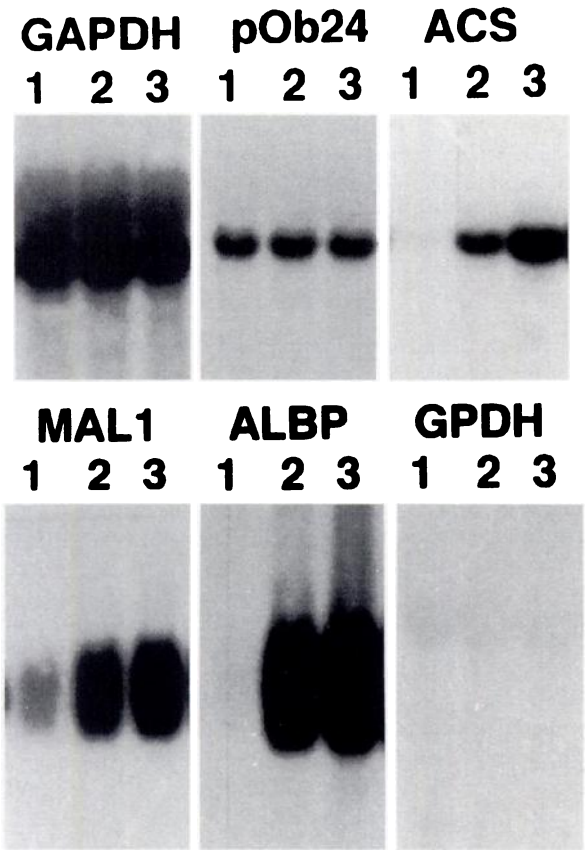

Fig. 1. Northern blot analysis of adipose-related mRNAs of Ob 1771 cells exposed to BRL 49653 or to 2-bromopalmitate. One-day postconfluent cells were maintained for 24 hr in standard medium, in the absence (*lanes 1*) or in the presence of 100 μM 2-bromopalmitate (*lanes 2*) or 100 μM BRL 49653 (*lanes 3*). RNA (20 μg/lane) was analyzed as described in Materials and Methods. The results are representative of five separate experiments.

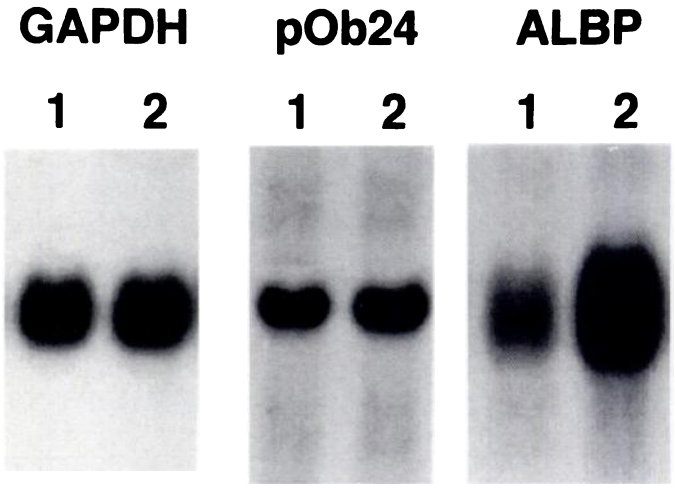

Fig. 2. Northern blot analysis of SVF cell RNA from adipose tissue exposed to BRL 49653. Cell culture protocol, preparation of the SVF, and RNA analysis were described in Materials and Methods. *Lanes 1*, control medium; *lanes 2*, medium supplemented with 0.8 μM BRL 49653.

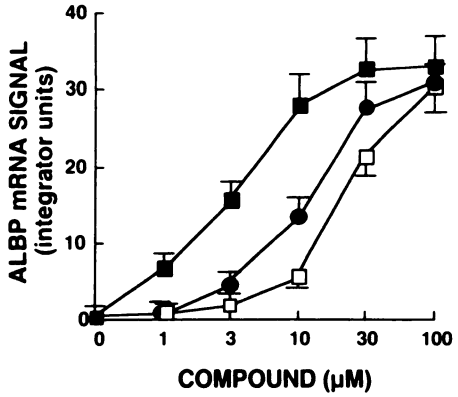

Fig. 3. Dose-response relationship for thiazolidinediones or 2-bromopalmitate effects on ALBP mRNA accumulation in preadipose Ob 1771 cells. One-day postconfluent cells maintained in standard medium were exposed for 24 hr to increasing concentrations of BRL 49653 (■), pioglitazone (●), or 2-bromopalmitate (□). RNA was analyzed as described in Materials and Methods. The ALBP mRNA signals were normalized to GAPDH mRNA signals and the results are expressed as the mean ± standard deviation of five separate experiments.

Effects of BRL 49653 on ALBP gene expression in rat adipose tissue. We showed previously that the ALBP gene was expressed at very low levels in cells from the SVF of rodent adipose tissue. This fraction contains preadipocytes, as demonstrated by the strong expression of pOb24/A2COL6 mRNA (22). To investigate the effects of thiazolidinedione on ALBP mRNA expression in SVF cells, pieces of adipose tissue from adult rats were incubated for 20 hr in chemically defined medium in the absence or presence of BRL 49653 (0.8 μM). Adipocytes and SVF cells were isolated after collagenase digestion and centrifugation. RNA from SVF cells was analyzed for the presence of ALBP mRNA, using GAPDH and pOb24/A2COL6 mRNAs as internal controls. As shown in Fig. 2, incubation with BRL 49653 did not change the amount of GAPDH and pOb24/A2COL6 mRNAs, whereas the signal for ALBP mRNA was strongly increased in treated adipose tissue. This observation indicates that BRL 49653 was effective in regulating ALBP gene expression in preadipocytes present in rat adipose tissue, as it is in mouse cells from preadipocyte clonal lines (Fig. 1). Furthermore, the effect in SVF cells did not require serum components.

Potency of BRL 49653, pioglitazone, and 2-bromopalmitate to induce ALBP mRNA accumulation. The accumulation of ALBP mRNA was studied as a function of inducer concentration after a 24-hr exposure of 1-day postconfluent cells. The potency of BRL 49653 was compared with that of pioglitazone and 2-bromopalmitate determined in the same series of cells. As shown in Fig. 3, the accumulation of ALBP mRNA was significant in cells exposed to 1 μM BRL 49653 and reached a plateau at 30 μM BRL 49653. The half-maximally effective concentration was estimated to be 3 μM. Pioglitazone exerted a similar positive effect on ALBP mRNA accumulation, but this occurred at higher concentrations than for BRL 49653. The effect of pioglitazone became significant at 3 μM and was maximal at 100 μM. In agreement with our previous data (10), 2-bromopalmitate appeared to be effective at concentrations ranging from 10 μM to 100 μM. Despite differences in half-maximally effective concentrations, i.e., ≈10 μM pioglitazone and ≈30 μM 2-bromopalmitate, compared with 3 μM BRL 49653, all compounds produced similar increases (70–80-fold) in ALBP mRNA content when present at maximally effective concentrations.

Time course of induction of ALBP mRNA by BRL 49653 and 2-bromopalmitate. The time course of ALBP

mRNA accumulation in cells exposed to BRL 49653 (100 μM) or 2-bromopalmitate (100 μM) is shown in Fig. 4A. In response to both compounds, the level of ALBP mRNA increased linearly with time from 0 to 24 hr of treatment and was maintained thereafter at levels that were 80-fold higher than those in control cells, provided that the inducer was present in the culture medium. Removal of BRL 49653 or 2-bromopalmitate after a 24-hr treatment resulted in a decrease in ALBP mRNA with a half-time of about 15 hr, which is consistent with the turnover rate of this mRNA in cultured cells (24). The kinetics of appearance and disappearance of ALBP mRNA in response to addition or removal, respectively, of BRL 49653 or 2-bromopalmitate appeared to be similar.

Additional experiments were performed to determine whether, as previously described for natural fatty acids (9) or 2-bromopalmitate (10), the induction of ALBP mRNA by thiazolidinediones was dependent upon *de novo* protein synthesis. For that purpose, 1-day postconfluent cells were exposed for 6 hr to BRL 49653 (30 μM), in the absence or presence of cycloheximide (18 μM). As shown in Fig. 4B, the induction of ALBP mRNA in response to BRL 49653 was totally abolished by cycloheximide, indicating that protein synthesis was required for this induction process.

Effects of BRL 49653 on 2-bromopalmitate- and dexamethasone-induced ALBP mRNA expression. To investigate further the relationships between thiazolidinediones and fatty acids in ALBP gene induction, the effects of treatment with combinations of BRL 49653 and 2-bromopalmitate were determined. One-day postconfluent cells were exposed for 24 hr to increasing concentrations of 2-bromopalmitate, in the absence or presence of 3 or 30 μM BRL 49653. When used at submaximally effective concentrations, i.e., 3 μM BRL 49653 and 30 μM 2-bromopalmitate, the two compounds were active in an additive manner to induce ALBP mRNA expression. In contrast, when a maximally effective concentration of a single compound was used, i.e., 30 μM BRL 49653 or 100 μM 2-bromopalmitate, addition of the second inducer did not result in any further increase of ALBP mRNA content (Fig. 5A). We reported previously that dexamethasone and fatty acids act in a more than additive manner to induce ALBP mRNA expression in preadipose cells (9). A similar pattern emerged when BRL 49653 was used as a substitute for fatty acids. Exposure to dexamethasone (1 μM) for 24 hr resulted in a 6-fold induction in ALBP mRNA level, whereas exposure to BRL 49653 (30 μM) led to a 75-fold induction. Treatment of the cells with a combination of the two inducers resulted in a 160-fold increase in the ALBP mRNA signal. This observation indicates that, even at maximally effective concentrations of BRL 49653, glucocorticoids are able to further increase ALBP gene expression.

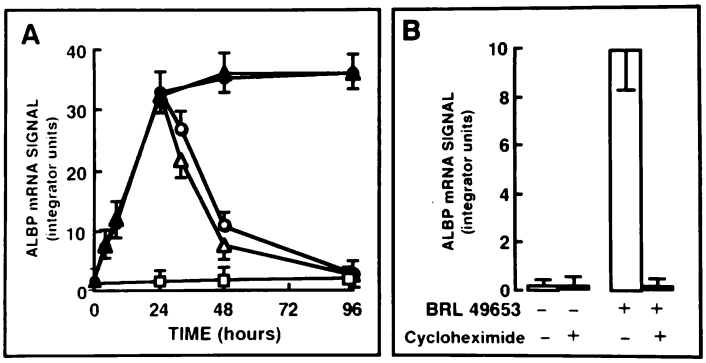

Fig. 4. Time course of induction of ALBP mRNA by BRL 49653 or 2-bromopalmitate. A, One-day postconfluent cells were maintained in standard medium in the absence (□) or presence of 100 μM BRL 49653 (●) or 2-bromopalmitate (▲). Removal of BRL 49653 (○) or 2-bromopalmitate (△) was performed with two washes and incubation in standard medium. The results are presented as in Fig. 2, from five separate experiments. B, The same cells as in A were exposed or not to 30 μM BRL 49653, in the absence or in the presence of 18 μM cycloheximide. Six hours later, RNA was isolated and analyzed as in Fig. 2. The results are the mean ± standard deviation of four separate experiments.

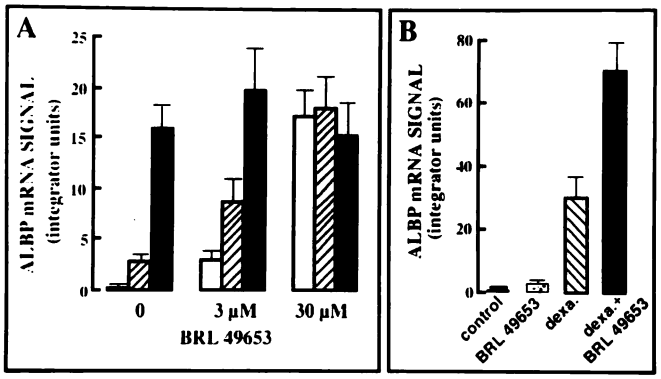

Fig. 5. Effects of BRL 49653 and other inducers of ALBP mRNA expression. A, One-day postconfluent cells were maintained for 24 hr in standard medium alone (□) or supplemented with 30 μM (▨) or 100 μM (■) 2-bromopalmitate, with the indicated concentrations of BRL 49653. B, The same cells as in A were maintained for 24 hr in control medium or were exposed to 1 μM dexamethasone (*dexa.*), to 30 μM BRL 49653, or to a combination of both agents. In A and B, RNA was analyzed as in Fig. 2 and the results are the mean ± standard deviation of four separate experiments.

BRL 49653 and fatty acid activation of the same nuclear receptor. We showed recently that the effects of fatty acids on gene expression in mouse preadipocytes could be mediated by a protein, named FAAR, that is a member of the superfamily of hormone nuclear receptors (15). As shown previously for other members of this family (11–14), we demonstrated that a hybrid receptor (hGR/FAAR) containing the DNA-binding domain from the hGR and the putative ligand-binding domain of FAAR is able to exhibit fatty acid-dependent transcriptional activation of the glucocorticoid-responsive MMTV promoter-driven CAT gene. Using the same *trans*-activation assay, we investigated the possible activation of the hGR/FAAR chimeric receptor by thiazolidinediones. As shown in Fig. 6, exposure to BRL 49653 of COS-1 cells transfected with the MMTV-CAT vector and the hGR/FAAR vector led, in a dose-dependent manner, to an increase in CAT activity. BRL 49653 was a more potent activator of the hGR/FAAR than was 2-bromopalmitate (26-fold induction of CAT activity with 10 μM BRL 49653, compared with 14-fold induction with 10 μM 2-bromopalmitate). Higher concentrations of both activators resulted in nearly complete disappearance of the stimulation of CAT activity. The reasons for this observation remain unclear but might be due to other receptors becoming activated and interfering with normal transcription. Control experiments, not reported here, showed that dexamethasone did not induce CAT activity in cells transfected with the MMTV-CAT vector and the hGR/FAAR vector, whereas BRL 49653 did not affect CAT activity in cells transfected with the MMTV-CAT vector only. Moreover, in cells transfected with the MMTV-CAT vector and the unmodified hGR, exposure to dexametha-

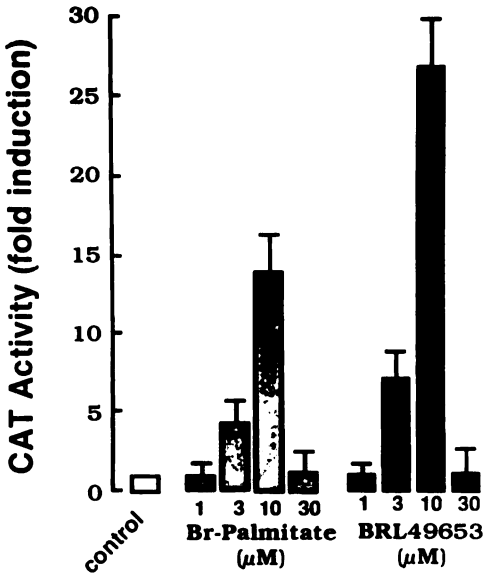

Fig. 6. Activation of hGR/FAAR hybrid receptor by 2-bromopalmitate and BRL 49653. COS-1 cells were transfected with the MMTV-CAT vector and the hGR/FAAR expression vector. Transfected cells were maintained in phenol red-free medium containing 8% activated charcoal-treated fetal calf serum and were exposed for 40 hr to either 2-bromopalmitate or BRL 49653 at the indicated concentrations. CAT enzyme activity was determined as described in Materials and Methods. Results were calculated by taking as 1 the value obtained in cells maintained in control medium and are presented as the mean ± standard deviation of triplicate transfections.

sone (0.1 μM) for 40 hr led to a 54-fold induction of CAT activity, whereas treatment with BRL 49653 did not lead to any significant induction of the enzymatic activity.

Evidence that expression of FAAR in 3T3-C2 fibroblasts confers BRL 49653-responsive gene expression. We have recently shown that the constitutive expression of FAAR in 3T3-C2 fibroblasts is sufficient to confer to the transfected cells the inducibility of the ALBP gene by fatty acids (15). To further investigate the relationship between the expression of FAAR and the regulation of gene expression by thiazolidinediones, the response to BRL 49653 was studied in 3T3-C2 fibroblasts stably transfected with either an empty expression vector (control transfected cells) or a FAAR expression vector (FAAR-transfected cells). As shown in Fig. 7A, control transfected cells expressed very small amounts of FAAR mRNA when maintained in standard medium (Fig. 7A, *lane 1*) or exposed for 24 hr to 30 μM BRL 49653 (Fig. 7A, *lane 2*). ALBP mRNA remained undetectable under both conditions. Similar patterns were observed with five independent cellular clones (data not shown). In FAAR-transfected cells, a very strong signal was obtained for the transfected form of FAAR mRNA (1.8 kilobases), which is easily distinguished from the endogenous form (3.1 kilobases) (Fig. 7A, *lanes 3* and *4*). When maintained in standard medium, FAAR-transfected cells did not express detectable amounts of ALBP mRNA (Fig. 7A, *lane 3*), whereas exposure to 30 μM BRL 49653 led to the emergence of the signal. Nearly identical results were obtained with three independent FAAR-transfected cellular clones (data not shown). The effects of various concentrations of BRL 49653 on ALBP gene expression in confluent control and FAAR-transfected cells are shown in Fig. 7B. ALBP mRNA remained undetectable in control transfected cells at all BRL 49653 concentrations, whereas a response to this compound in FAAR-transfected cells was detectable at 1 μM BRL 49653 and reached a plateau at 30 μM BRL 49653; the half-maximal effect was observed at ≈10 μM. Taken together, these observations indicate that, as previously described for fatty acids, the thiazolidinedione BRL 49653 acts as an activator of the nuclear receptor FAAR.

Discussion

It is well established that the improvements in insulin sensitivity produced by the thiazolidinedione pioglitazone are correlated with transcriptional activation of ALBP in adipocytes (5, 7). In the present report, we demonstrate that pioglitazone and BRL 49653 elicit a striking activation of genes encoding proteins directly involved in fatty acid metabolism, i.e., ACS and the fatty acid-binding proteins ALBP and MAL1 (Fig. 1). This effect of thiazolidinediones is neither related to indirect actions through serum components nor confined to established cell lines, because it occurs in preadipocytes from rat adipose tissue maintained in serum-free medium (Fig. 2). This observation is reminiscent of fatty acid effects in preadipose cells (8–10, 25). Several lines of evidence support the hypothesis that fatty acids and BRL 49653 exert their effects through identical mechanisms. (i) The induction process in Ob 1771 cells in response to BRL 49653 is kinetically identical to that

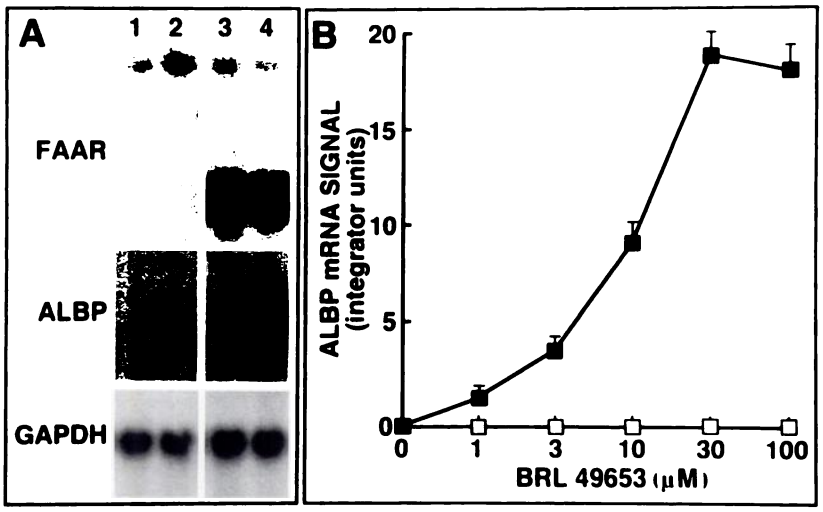

Fig. 7. Effects of BRL 49653 on gene expression in FAAR-transfected 3T3-C2 fibroblasts. A, One-day postconfluent control transfected cells (*lanes 1* and *2*) or FAAR-transfected cells (*lanes 3* and *4*) were maintained for 24 hr in standard medium alone (*lanes 1* and *3*) or supplemented with 30 μM BRL 49653 (*lanes 2* and *4*). RNA (20 μg/lane) was analyzed as described in Materials and Methods. The results are representative of three separate experiments. B, One-day postconfluent control transfected cells (□) or FAAR-transfected cells (■) maintained in standard medium were exposed for 24 hr to increasing concentrations of BRL 49653. RNA was analyzed as in Fig. 3 and the results are expressed as the mean ± standard deviation of three separate experiments.

in response to fatty acids, occurring within hours and reaching a plateau after 1 day. (ii) As previously demonstrated for fatty acids, this phenomenon is fully reversible upon removal of inducer from the medium. (iii) Ongoing protein synthesis is required for the effects of both compounds (Fig. 4 and Refs. 9 and 10). (iv) The additive effects of BRL 49653 and fatty acids are lost when a maximally effective concentration of each is used, whereas both BRL 49653 and fatty acids act synergistically with dexamethasone (Fig. 5 and Refs. 9 and 10).

We postulate that thiazolidinediones interact with a nuclear receptor that belongs to the steroid/thyroid hormone receptor superfamily. A subclass of these receptors, called PPARs, have been implicated as nuclear targets for fibrates and fatty acids (11–14), and we have recently described the identification in preadipose cells of a member of the family that is activated by fatty acids. This FAAR protein is expressed in various tissues, i.e., adipose tissue, intestine, muscle, heart, brain, and lung, and is induced during the process of adipose cell differentiation. In this paper, we present some experimental data supporting the hypothesis that FAAR is involved as a nuclear mediator of the transcriptional effects of thiazolidinediones. The thiazolidinedione BRL 49653 acts as a potent activator of the hGR/FAAR hybrid receptor in transfected COS-1 cells (Fig. 6). Furthermore, transfection of 3T3-C2 fibroblasts with a FAAR expression vector confers BRL 49653 inducibility of ALBP gene expression (Fig. 7).

BRL 49653 is more potent than pioglitazone in inducing ALBP gene expression. BRL 49653 is also more potent than pioglitazone in improving insulin sensitivity *in vivo* (16). Thiazolidinediones exert their effects at lower concentrations than those required for 2-bromopalmitate, which is itself a better inducer than natural fatty acids (10). This difference between BRL 49653 and 2-bromopalmitate is not due to changes in medium fatty acid concentration, because we have demonstrated that 2-bromopalmitate is not metabolized by preadipose cells (10), but could be a reflection of different affinities of the molecules for albumin, leading to various concentrations of unbound compound in the medium. Alternatively, this shift of effective concentrations could be a reflection of a higher concentration of thiazolidinediones not bound to MAL-1 in the cytosol and, in turn in the nucleus, and/or a better affinity of FAAR for BRL 49653. Because fatty acids and thiazolidinediones are structurally diverse molecules, this raises the issue of the specificity of ligand-FAAR interactions, but a similar lack of specificity has already been observed for other PPARs, which are activated by a broad spectrum of molecules including fatty acids, fibrates, and phtalates (11–14, 26). Possible explanations for this lack of specificity could be that these molecules are not the actual ligands for the receptor and/or that these molecules affect the phosphorylation level of the nuclear receptor in a process similar to that described for some members of the steroid/thyroid hormone nuclear receptor family (27–30). Interestingly, fibrates clearly act as weaker inducers, compared with fatty acids and thiazolidinediones, of the hGR/FAAR chimeric receptor in COS-1 cells as well as of FAAR in 3T3-C2-transfected cells (15). This suggests that FAAR specificity is different from that of other members of the PPAR subfamily, which are fully responsive to fibrates (11–14). This is also the case for the adipose-specific form of PPAR-γ2 recently described as being involved as a transcriptional mediator of fatty acid and fibrate activation of ALBP gene expression (31). PPAR-γ2 interacts with a distal element of the ALBP promoter, called the ARE, that previously was identified as being crucial for tissue-specific expression of the gene (32) and more recently has been implicated in the pioglitazone-induced transcriptional activation of the ALBP gene (33). Because ARE is very similar to peroxisomal proliferator-responsive elements and we demonstrated that FAAR binds to the peroxisomal proliferator-responsive element from the ACS gene, (15). it is tempting to speculate that FAAR could interact also with ARE to regulate ALBP gene expression.

Whatever the exact mechanisms of FAAR action, the data herein demonstrate that, in preadipose cells, thiazolidinediones activate a powerful fatty acid-like activity controlling the expression of genes directly involved in fatty acid metabolism. Fatty acids and BRL 49653 appear to activate transcriptional expression of these genes by means of the same nuclear receptor. It is possible that such effects of thiazolidinediones on gene expression in preadipose cells may contribute to the beneficial actions of this class of drugs on insulin sensitivity in rodent models of non-insulin-dependent diabetes mellitus. A better understanding of the mechanism of thiazolidinedione action awaits more complete knowledge of the physiological role of the FAAR, including determination of the mechanism of activation, characterization of the physiological ligand, and identification of DNA-binding motifs in the promoter regions of target genes.

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