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Glucocorticoids Repress Induction by Thiazolidinediones, Fibrates, and Fatty Acids of Phosphoenolpyruvate Carboxykinase Gene Expression in Adipocytes

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Abstract Phosphoenolpyruvate carboxykinase (PEPCK) exerts a glyceroneogenic function in adipocytes in which transcription of its gene is increased by unsaturated fatty acids and fibrates. We used cultured rat adipose tissue fragments and 3T3-F442A adipocytes to show that the antidiabetic thiazolidinedione BRL 49653, a ligand and an activator of the γ isoform of peroxisome proliferator activated receptors (PPARγ), is a potent inducer of PEPCK mRNA. In 3T3-F442A adipocytes, the effect of BRL 49653 is rapid and concentration dependent, with a maximum reached at 1 μM and a half-maximum at 10–100 nM. PEPCK mRNA is similarly induced by the natural ligand of PPARγ, the 15-deoxy-Δ^{12-14} prostaglandin J2. These observations strongly suggest that PPARγ is a primary regulator of PEPCK gene expression in adipocytes. Dexamethasone at 10 nM repress induction of PEPCK mRNA by 1 μM BRL 49653, 0.32 mM oleate, or 1 mM clofibrate, in a cycloheximide-independent manner. The antiglucocorticoid RU 38486 prevents dexamethasone action, demonstrating involvement of the glucocorticoid receptor. Stable transfectants of 3T3-F442A adipocytes bearing −2100 to +69 base pairs of the PEPCK gene promoter fused to the chloramphenicol acetyltransferase (CAT) gene respond to 1 μM BRL 49653 or 1 mM clofibrate by a large increase in CAT activity, which is prevented by the simultaneous addition of 10 nM dexamethasone. Hence, in adipocytes, glucocorticoids act directly through the 5′-flanking region of the PEPCK gene to repress, in a dominant fashion, the stimulation of PEPCK gene transcription by thiazolidinediones and fibrates. J. Cell. Biochem. 68:298–308, 1998.

Key words: PEPCK; adipocytes; transcription; fatty acids; fibrates

Phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32; PEPCK), a key enzyme in liver gluconeogenesis and in adipose tissue glyceroneogenesis, is under multiple hormonal and nutritional control. There is no known post-translational regulation of PEPCK. Hence, modifications of enzymatic activity reflect changes in PEPCK mRNA concentration. Cyclic adenosine monophosphate (cAMP) and retinoic acid stimulate PEPCK mRNA synthesis by a process that does not involve protein synthesis, both in hepatic cells (hepatoma cell lines and primary hepatocytes) and in adipocytes [Antras-Ferry et al., 1994a; Franckhauser et al., 1995; Granner and Andreone 1985; Lamers et al., 1982; Lucas et al., 1991]. Glucocorticoids, however, oppositely regulate PEPCK mRNA in adipocytes and hepatic cells [Franckhauser et al., 1994, 1995; Franckhauser-Vogel et al., 1997a,b; Granner et al., 1991; Meyuhas et al., 1976; Nechushtan et al., 1987]. In the latter cells, induction of the gene by glucocorticoids is coherent with the role played by these hormones in gluconeogenesis. In adipocytes, glucocorticoids repress PEPCK gene transcription [Franckhauser et al., 1995] and inhibit stimulation by cAMP, retinoic acid, and fibrates [Franckhauser et al., 1994, 1995; Franckhauser-Vogel et al., 1997]. Such regulation is in accordance with the large increase of circulating glucocorticoids in response to stress, a situation requiring energy supply to the blood. Indeed, the postulated role of PEPCK is to provide

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adipocytes with glycerol 3-phosphate needed for fatty acid esterification, thereby restraining fatty acid output during lipolysis. Inhibition of PEPCK is therefore beneficial to fatty acid supply to the blood.

We used 3T3-F442A adipocytes to demonstrate that unsaturated fatty acids are strong inducers of PEPCK mRNA [Antras-Ferry et al., 1994b, 1995]. Stimulation by fatty acids is transcriptional, at least in part, and involves the region of the gene between −2100 and +69 base pairs (bp) relative to the transcription start site [Antras-Ferry et al., 1995]. Among the potential mechanisms by which fatty acids can modulate gene transcription, a model involving binding and activation of the peroxisome proliferator-activated receptor (PPAR) class of nuclear receptors has been proposed [Bocos et al., 1995; Forman et al., 1997; Kliewer et al., 1997; Krey et al., 1997; Lemberger et al., 1996; Wahli et al., 1995]. Heterodimers of PPARs with retinoid X receptors (RXR) recognize imperfect versions of a direct repeat of the AGGTCA consensus sequence separated by one nucleotide (DR1) in target genes [Lemberger et al., 1996; Mangelsdorf and Evans, 1995]. Three isoforms of PPARs (α, β = δ, γ) and of RXRs (α, β, γ) have been isolated [Lemberger et al., 1996]. A variant of PPARγ, the PPARγ2, shows adipose-selective expression, heterodimerizes with RXRα, and is strongly induced at an early step of adipocyte differentiation [Brun et al., 1996; Tontonoz et al., 1994a,b]. The PEPCK gene promoter-regulatory region contains two DR1-like sequences able to bind a PPARγ2/RXRα heterodimer in a gel-shift assay [Tontonoz et al., 1995]. One (PCK2) is located between −999 and −987 bp. The other, named AF1 element or RARE1, is between −451 and −433 bp [Lucas et al., 1991; Scott et al., 1996; Tontonoz et al., 1995]. Moreover, the −2086 to −460 bp of the PEPCK gene promoter can be activated by the arachidonic analogue ETYA or by linoleic acid in a transactivation assay in which PPARγ2 and RXRα are co-overexpressed in NIH-3T3 non-adipose cells [Tontonoz et al., 1995].

The potent antidiabetic thiazolidinediones are selective ligands and activators of PPARγ [Lehmann et al., 1995; Willson et al., 1996]. The search for the natural PPARγ ligand has led to the discovery that 15-deoxy-Δ^{12-14}-prostaglandin J2 (15d-PGJ2), an arachidonic acid metabolite of the prostaglandin D2-J2 series, is able to compete specifically with the labeled thiazolidinedione BRL 49653 for binding to PPARγ [Forman et al., 1995; Kliewer et al., 1995]. Therefore, we decided to investigate the effect that thiazolidinediones and 15d-PGJ2 would have on PEPCK gene expression in adipocytes. Here we demonstrate that BRL 49653 and 15d-PGJ2 are potent inducers of PEPCK gene expression in adipocytes. We also show that glucocorticoids repress stimulation by thiazolidinediones, fatty acids, and fibrates by a process involving the 5′-flanking region of the gene.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM) and geneticin G418 were from Gibco BRL. Fetal bovine serum (FBS) was from Boehringer Mannheim. [α-^{32}P]-dCTP, [^{14}C]chloramphenicol, Hybond-N$^+$ blotting membranes, and X-ray films were from Amersham (Braunschweig, Germany). The random priming kit was from Stratagene (La Jolla, CA). 15-Deoxy-Δ^{12-14} prostaglandin J2 was from Cayman Chemicals (Ann Arbor, MI). Essentially fatty acid free bovine serum albumin (BSA) (Cat. # A-6003), fatty acids, clofibric acid (Cat. # C-7142), and all other products were purchased from Sigma (St. Louis, MO).

Culture of Adipose Tissue Explants

Six-week-old male Wistar rats were killed by decapitation. Periepididymal fat pads were quickly removed, cut in two pieces, and washed in glucose-free DMEM containing 10 mM pyruvate, 1 mM lactate, and 40 µM BSA. This medium was referred to as Medium 1. Tissues were cultured in 60 mm plates in Medium 1 at 37°C in a humidified atmosphere of 10% CO_2–90% air.

Cell Culture and Treatment

3T3-F442A cells and PEPCK-CAT stable transfectants were cultured in DMEM containing glucose (25 mM) at 37°C in a humidified atmosphere of 10% CO_2–90% air. They were grown and differentiated in 10% newborn calf serum (Gibco, Grand Island, NY), penicillin (200 IU/ml), streptomycin (50 mg/L), biotin (8 mg/L), and pantothenate (4 mg/L). At a confluence of the cells, newborn calf serum was changed to FCS (10%), and insulin (20 nM) was added to the medium to favor triglyceride accumulation. Medium was changed every 2–3 days

for 8 days. Experiments were carried out on mature adipose cells (8 days after confluence). At 24 h before RNA extraction, cells were maintained in BSA-free Medium 1. Treatments with effectors were performed for 4–24 h in Medium 1.

RNA Extraction and Analysis

RNA from tissue fragments and from cultured cells was extracted by the method of Chirgwin et al. [1979] and of Chomczynski and Sacchi [1987], respectively. Total RNA was electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde and blotted onto a nylon membrane. The integrity and relative amounts of RNA were assessed by Methylene Blue staining. Prehybridization and hybridization of the blots were carried out according to Yang et al. [1993]. Membranes were hybridized overnight at 65°C in 0.27 M NaCl, 1.5 mM EDTA, 15 mM sodium phosphate (pH 7.7) containing 7% sodium dodecyl sulfate (SDS), 10% polyethylene glycol (PEG) 6000, 250 µg/ml sonicated salmon sperm DNA, 250 µg/ml heparin, and 10^6 cpm/ml of cDNA labeled with [α^{32}P]-dATP by random priming, according to the manufacturer's recommendations. Membranes were washed twice for 15 min at room temperature in 2× SSC (1× SSC is 0.3 M NaCl, 0.03 M sodium citrate, pH 7), 0.1% SDS, and then for 30 min at 60°C with 0.1× SSC, 0.1% SDS. Specific cDNA probes used were PC 116, a rat PEPCK cDNA fragment [Beale et al., 1985], and ACT-1, a mouse β-actin cDNA fragment [Spiegelman et al., 1983], used as a control. The PEPCK mRNA signal was quantified by scanning densitometry and was corrected for differences in RNA loading by comparison with the signals generated by the β-actin cDNA probe.

Stable Transfections and CAT Activity Determination

The generation of the FL15 and FL18 stable transfectants has been described elsewhere [Antras-Ferry et al., 1994; Franckhauser et al., 1994a, 1995]. Briefly, 2 µg pSV2-NEO, the expression of which confers resistance to the antibiotic geneticin, G418, was co-transfected with 20 µg pPL1-CAT (−2100 to +69 of the PEPCK gene promoter fused to the chloramphenicol acetyltransferase gene) by calcium phosphate/DNA co-precipitation onto exponentially growing 3T3-F442A preadipocytes (10^5 cells/100-mm plate). Cells were then treated essentially as described previously [Franckhauser et al., 1994]. After 2 weeks of exposure to G418 (0.4 mg/ml), colonies resistant to the antibiotic were isolated and analyzed as described [Franckhauser et al., 1994]. Preparation of cell homogenates for CAT assays was performed as detailed by Forest et al. [1990]. The method of Seed and Sheen [1988] was used for CAT activity determination. One unit of CAT converts 1 µmole of chloramphenicol to butyryl-chloramphenicol per minute at pH 7.8 and 37°C.

RESULTS

Activators of PPARγ Stimulate PEPCK mRNA in Cultured Adipose Tissue Fragments and in 3T3-F442A Adipocytes

We previously showed that long-chain unsaturated fatty acids and clofibrate stimulate PEPCK gene expression in 3T3-F442A adipocytes [Antras-Ferry et al., 1995]. At various levels, adipocytes express the PPARα, δ, and γ isoforms, which can be the target to fatty acids and fibrates [Braissant et al., 1996; Tontonoz et al., 1994a,b]. Among these isoforms, PPARγ2 is expressed at the highest level [Tontonoz et al., 1994a,b]. We wondered whether PPARγ ligands would stimulate PEPCK mRNA in adipocytes. To address this question, we first isolated perirenal adipose tissue fragments from fed rats and analyzed PEPCK mRNA content by Northern blots (Fig. 1, t0). These fragments were then cultured for 4 h with or without 1 µM isoprenaline (IPR), a β-adrenergic receptor agonist or 1 µM BRL 49653, a powerful PPARγ activator. As shown in Figure 1, PEPCK mRNA

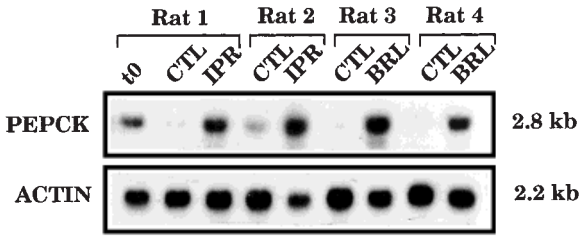

Fig. 1. Effect of isoprenaline and of BRL 49653 on PEPCK mRNA in cultured adipose tissue fragments. Adipose tissue fragments from four rats were isolated as described under Materials and Methods. Part of the biological material was immediately frozen in liquid nitrogen for the time 0 of the experiment (t0). The other part was cultured for 4 h in the absence (CTL) or presence of 1 µM isoprenaline (IPR), or 1 µM BRL 49653 (BRL), then frozen in liquid nitrogen before RNA extraction and analysis. PEPCK and actin mRNAs were revealed by sequential hybridization of the same blot with the corresponding probes.

decreased with time when fragments were cultured in the basal condition (Fig. 1, CTL). In the presence of IPR or BRL 49653, PEPCK mRNA was increased either slightly or strongly, when compared, respectively, to the signal obtained before (Fig. 1, t0) or after 4 h in culture (Fig. 1, IPR, BRL). The actin mRNA remained similar, whatever the treatment, showing that IPR and BRL 49653 specifically altered PEPCK gene expression (Fig. 1).

Because the PEPCK mRNA signal was unstable in cultured adipose tissue fragments, we decided to use 3T3-F442A adipocytes as a stable model for the study of PEPCK gene regulation [Forest et al., 1997]. In these cells, 1 μM BRL 49653 stimulated PEPCK mRNA specifically six- to eightfold in 4 h (Fig. 2A). This stimulation was maintained for at least 24 h (Fig. 2A). We selected a treatment time of 4 h for determining the concentration dependence of the BRL

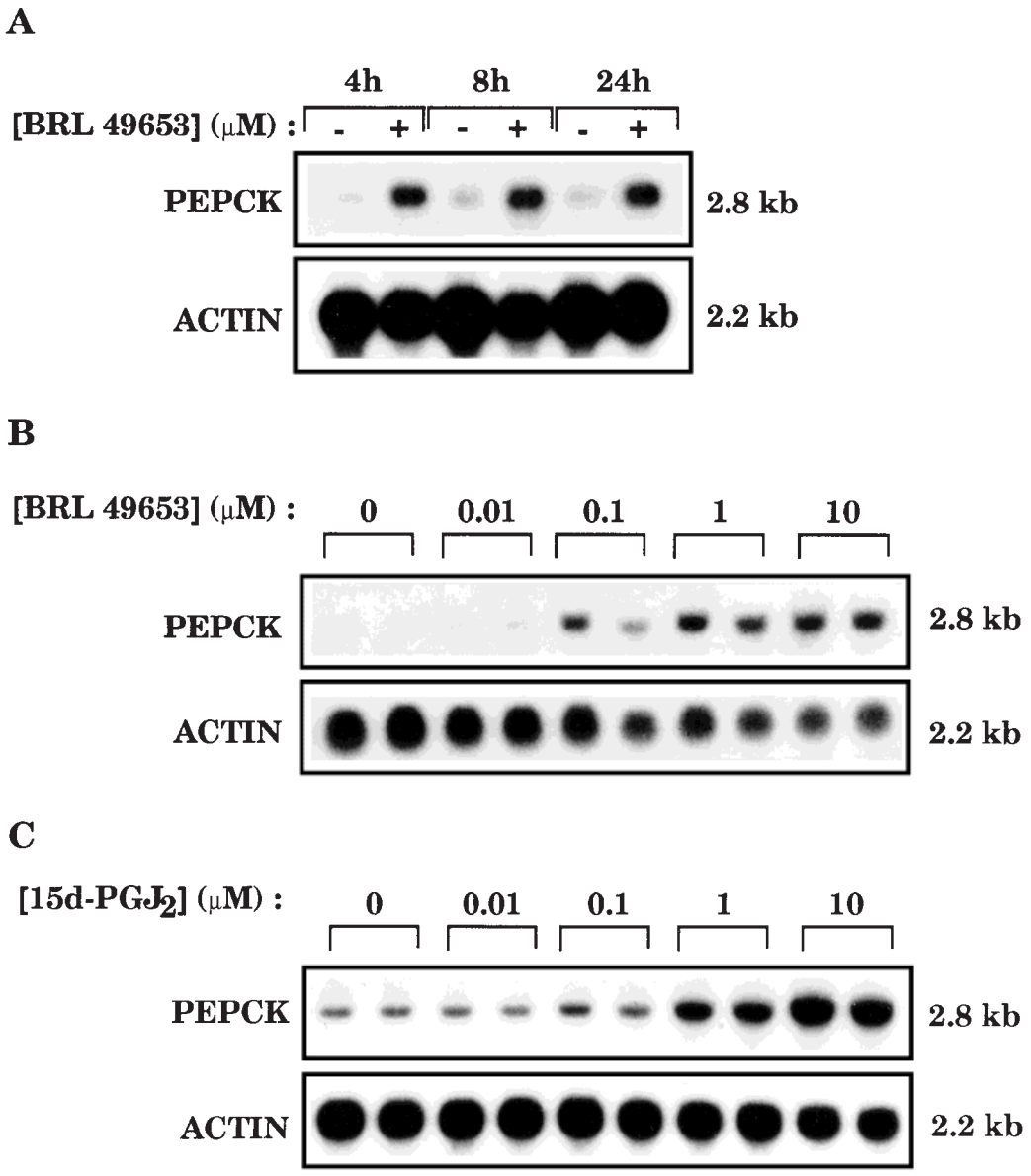

Fig. 2. Modulation of PEPCK mRNA level by BRL 49653 or 15-deoxy d12_14-prostaglandin J2 in 3T3-F442A adipocytes. RNA from 3T3-F442A adipocytes was isolated and analyzed by Northern blot with PEPCK and actin probes. **A:** Time course of the effect of 1 μM BRL 49653. CTL, control cells. **B,C:** Effect of a 4-h treatment of the cells with increasing concentrations of BRL 49653 (B) or of 15-deoxy d12-14-prostaglandin J2 (15d-PGJ2) (C). For each concentration, RNA from duplicate dishes has been used and probed. PEPCK and actin mRNAs were revealed by sequential hybridization of the same blot with the corresponding probes.

49653 effect (Fig. 2B). The increase in PEPCK-mRNA was dose dependent, with a maximum reached at 1 μM and a half-maximum effect (Ec50) obtained at 10–100 nM. Such a concentration was quite similar to the reported Kd of BRL 49653 for PPARγ and to the Ec50 for reporter gene expression in transactivation assay [Lehmann et al., 1995; Willson et al., 1996].

The natural specific activator of PPARγ was reported to be the 15-deoxy-Δ^{12-14} prostaglandin J2 (15d-PGJ2) [Forman et al., 1995; Lehmann et al., 1995]. We wondered whether this prostaglandin would mimic the action of BRL 49653 on PEPCK gene expression. Indeed, 4-h treatment of 3T3-F442A adipocytes with 15d-PGJ2 induced a dose-dependent increase in PEPCK mRNA, with a maximum attained at 10 μM (Fig. 2C).

Glucocorticoids Antagonize Activation of PEPCK mRNA by PPAR Activators, Fatty Acids, and Isoprenaline in 3T3-F442A Adipocytes

Glucocorticoids are strong repressors of PEPCK gene expression in adipocytes [Franckhauser et al., 1995; Meyuhas et al., 1976; Nechushtan et al., 1987]. In order to determine whether β-agonists and BRL 49653 cooperated to stimulate PEPCK gene expression and whether glucocorticoids antagonized these stimulations, we treated 3T3-F442A adipocytes with 1 μM isoprenaline, 1 μM BRL 49653 or 0.1 μM dexamethasone alone or in combination; 4 h later, we extracted total RNA and analyzed PEPCK mRNA by Northern blot. Isoprenaline, BRL 49653, or both induced PEPCK mRNA to the same extent (about sevenfold) (Fig. 3A), while dexamethasone inhibited these stimulations at least partially (Fig. 3). In an attempt to determine whether BRL 49653 and isoprenaline intracellular pathways could interact to alter PEPCK gene expression, we used submaximal concentrations of both effectors, i.e., 0.1 μM BRL 49653 (Fig. 2) and 0.01 μM isoprenaline (Fig. 3), alone or in combination. As shown in Table I, the combination of both effectors induced an additive action, suggesting that BRL 49653 and isoprenaline followed either a single, or two independent, noninteracting pathways.

The monounsaturated fatty acid oleate and clofibrate are potent inducers of PEPCK mRNA and gene transcription [Antras-Ferry et al., 1995]. We treated 3T3-F442A adipocytes for 4 h with 0.32 mM oleate or 1 mM clofibrate in the presence or absence of 0.01 μM dexamethasone. PEPCK mRNA was increased five- to six-fold by oleate or clofibrate; dexamethasone inhibited these inductions almost totally (Fig. 3B). Isoprenaline, 1 μM, did not significantly modify the magnitude of oleate or clofibrate effect, and dexamethasone reduced the stimulations obtained by combined actions of the effectors (Fig. 3B). Hence, dexamethasone inhibited PEPCK mRNA inductions in a dominant fashion in adipocytes.

We next focused on the mechanism of thiazolidinedione, fibrate, and glucocorticoid effects. Cells were exposed to the antiglucocorticoid RU 38486 and either BRL 49653 or clofibrate in the presence or absence of dexamethasone, in an effort to determine whether these effects involved the glucocorticoid receptor. RU 38486 affected neither PEPCK mRNA basal level nor BRL 49653 (Fig. 4A) or clofibrate stimulation (Fig. 4B). In contrast, RU 38486 severely blunted the repression by dexamethasone of BRL 49653 or clofibrate induction. These results strongly suggested that the glucocorticoid receptor was mediating dexamethasone action.

BRL 49653, Clofibrate, and Dexamethasone Effects Are Independent of Protein Synthesis and Involve the Regulatory Region of the PEPCK Gene

In order to determine whether the actions of BRL 49653, clofibrate, and dexamethasone were direct, we preincubated cells for 30 min with the protein synthesis inhibitor cycloheximide; the effectors were then added to the cells for 4 h and PEPCK mRNA content analyzed. As we showed previously [Franckhauser-Vogel et al., 1997a], cycloheximide did not significantly alter basal PEPCK mRNA, whereas it abolished the dexamethasone-induced decrease (Fig. 5A,B). Cycloheximide affected neither BRL 49653 nor clofibrate induction, nor did it prevent dexamethasone repression of these inductions, showing that these effects were direct (Fig. 5A,B).

We showed previously that clofibrate action was transcriptional and mediated by the 5′-flanking region of the PEPCK gene [Antras-Ferry et al., 1995; Franckhauser-Vogel et al., 1997b]. To confirm this result and to determine whether the thiazolidinedione and glucocorticoid effects were transcriptional, we used the previously described 3T3-F442A stable transfectants, FL15 and FL18, bearing −2100 to +69 bp of the PEPCK gene promoter region fused to the chloramphenicol acetyltransferase (CAT)

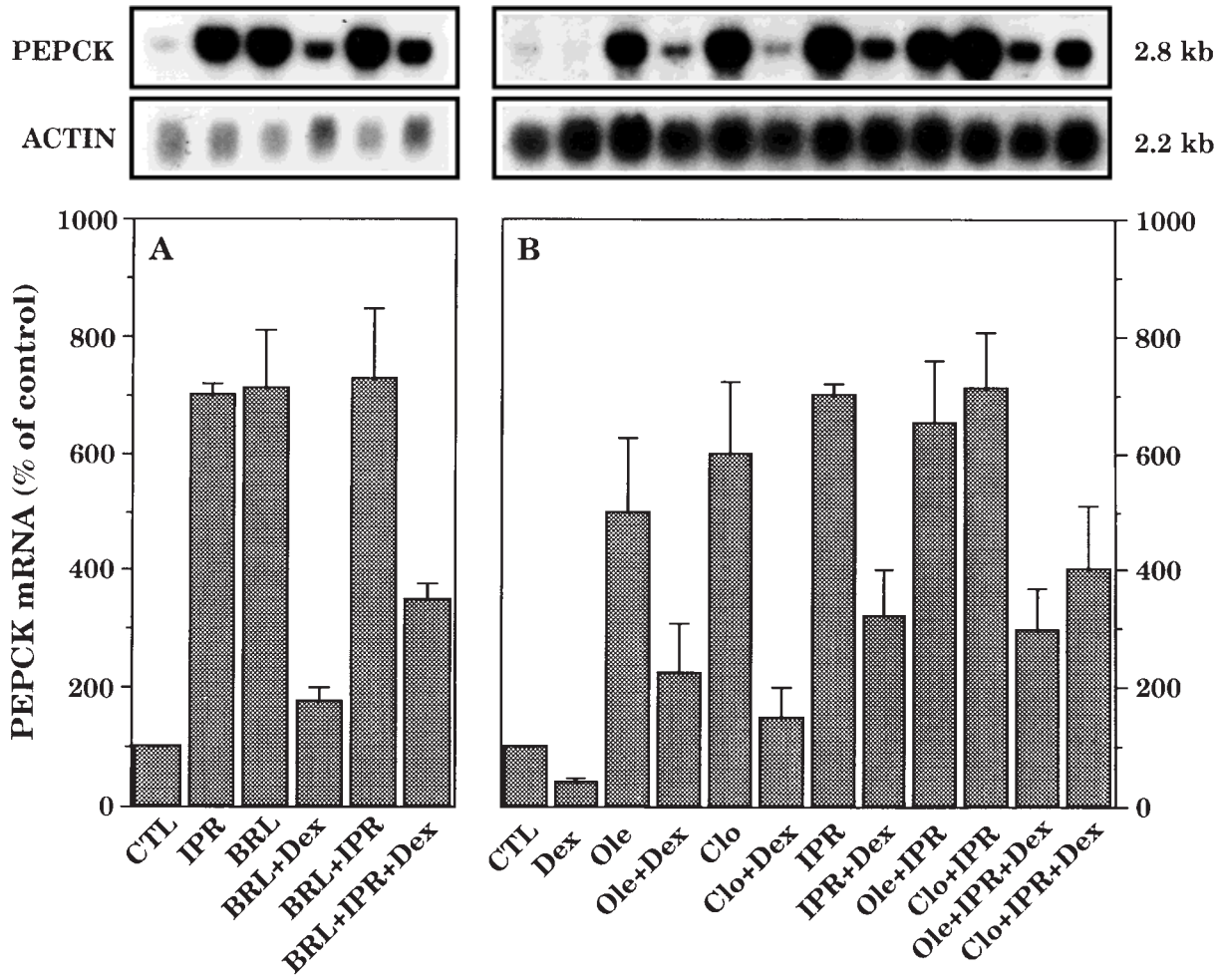

Fig. 3. Effects of isoprenaline, BRL 49653, dexamethasone, oleate, and clofibrate on PEPCK mRNA in 3T3-F442A adipocytes. 3T3-F442A adipocytes were treated with 1 µM isoprenaline (IPR) and 1 µM BRL 49653 (BRL) **(A)** or with 0.1 µM dexamethasone (Dex), 320 µM oleate (Ole), and 1 mM clofibrate (Clo) **(B),** either alone or in combination, for 4 h before RNA extraction and analysis. **Top:** Results of typical autoradiograms obtained with blots hybridized sequentially with the PEPCK and actin probes are presented. **Bottom:** Data obtained by scanning densitometry, was normalised for differences in RNA loading by using the actin mRNA signal and expressed as the percent of the PEPCK signal from control (CTL), untreated cells. Each value is the mean of data collected from two independent experiments with duplicate dishes.

TABLE I. Analysis of the Combined Effects of BRL 49653 and Isoprenaline at Submaximal Concentrations on PEPCK mRNA in 3T3-F442A Adipocytes*

BRL 49653, 0.1 µM	−	+	−	+
IPR, 0.01 µM	−	−	+	+
PEPCK mRNA (% of control)	100	495	236	605

*3T3-F442A adipocytes were treated with 0.1 µM BRL 49653 and 0.01 µM isoprenaline either alone or in combination for 4 h before RNA extraction and analysis. For each condition, RNA from duplicate dishes was extracted and probed. PEPCK and actin mRNAs were revealed by sequential hybridization of the same blot with the corresponding probes. Results are expressed as the percentage of the PEPCK signal from control untreated cells.

gene [Franckhauser et al., 1994]. Cells were allowed to differentiate into adipocytes then treated for 15 h with 0.1 µM dexamethasone, 1 mM clofibrate, or 1 µM BRL 49653, alone or in combination, before CAT was measured. Variations in CAT activity directly reflect the hormonal modulation of the PEPCK promoter. As shown in Figure 6, clofibrate stimulated CAT activity about two- and fourfold, while BRL 49653 was more potent with a 33- and ninefold increase, for FL15 and FL18, respectively. Dexamethasone did not affect basal CAT but repressed BRL 49653 and clofibrate effects almost totally in both transfectants. Hence, BRL 49653 and clofibrate clearly enhanced transcrip-

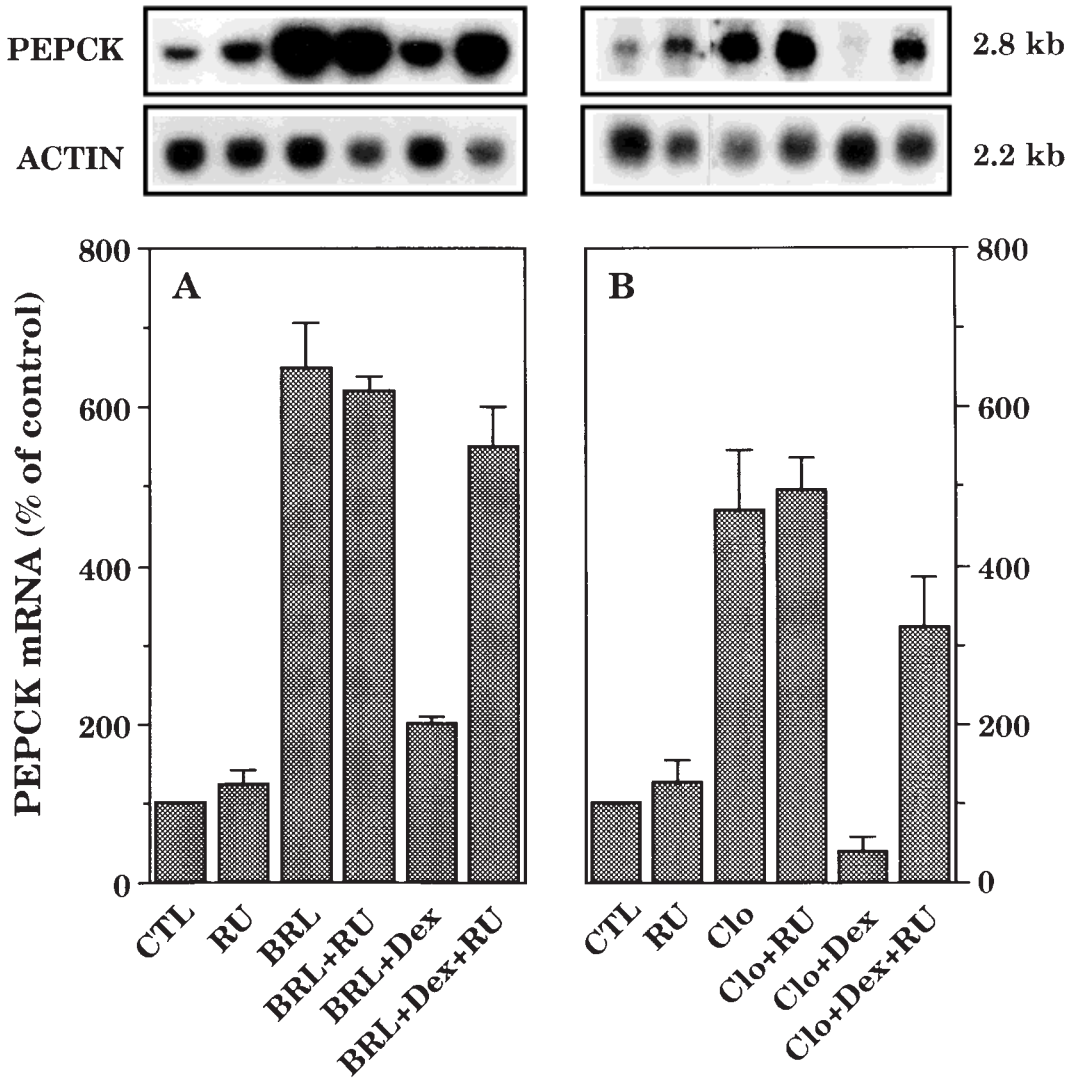

Fig. 4. Effect of RU 38486 on the modulation by BRL 49653, clofibrate, or dexamethasone of PEPCK mRNA in 3T3-F442A adipocytes. 3T3-F442A adipocytes were treated for 4 h with 0.1 µM RU 38486 (RU) **(A,B),** 1 µM BRL 49653 (BRL) **(A),** 1 mM clofibrate (Clo) **(B),** in the presence or absence of 0.01 µM dexamethasone (Dex) before RNA extraction and analysis. **Top:** Results of typical autoradiograms obtained with blots hybridized sequentially with the PEPCK and actin probes. **Bottom:** Data obtained by scanning densitometry were normalised for differences in RNA loading by using the actin mRNA signal and expressed as the percentage of the PEPCK signal from control (CTL), untreated cells. Each value is the mean of data collected from two independent experiments with duplicate dishes.

tion of the PEPCK gene and acted through the promoter region of the gene, while glucocorticoids inhibited these stimulations.

DISCUSSION

The thiazolidinedione BRL 49653 is a potent inducer of PEPCK gene expression in 3T3-F442A adipocytes. The effect of BRL 49653, like that of clofibrate [Forest et al., 1997], is rapid, long-lasting, and independent of protein synthesis and involves the 5′-flanking of the PEPCK gene between −2100 and +69 bp. In contrast to clofibrate, BRL 49653 acts within a low range of concentrations (Fig. 2), in good agreement with the reported affinity of the drug for PPARγ [Lehmann et al., 1995; Willson et al., 1996]. PEPCK gene expression is also very sensitive to the natural and specific ligand of PPARγ, the 15d-PGJ2 (Fig. 2). These results strongly suggest the involvement of the PPARγ isoform in the action of thiazolidinediones on the PEPCK gene in adipocytes. This hypothesis is consistent with PPARγ as the predominant PPAR isoform in adipocytes. We have no evidence of the

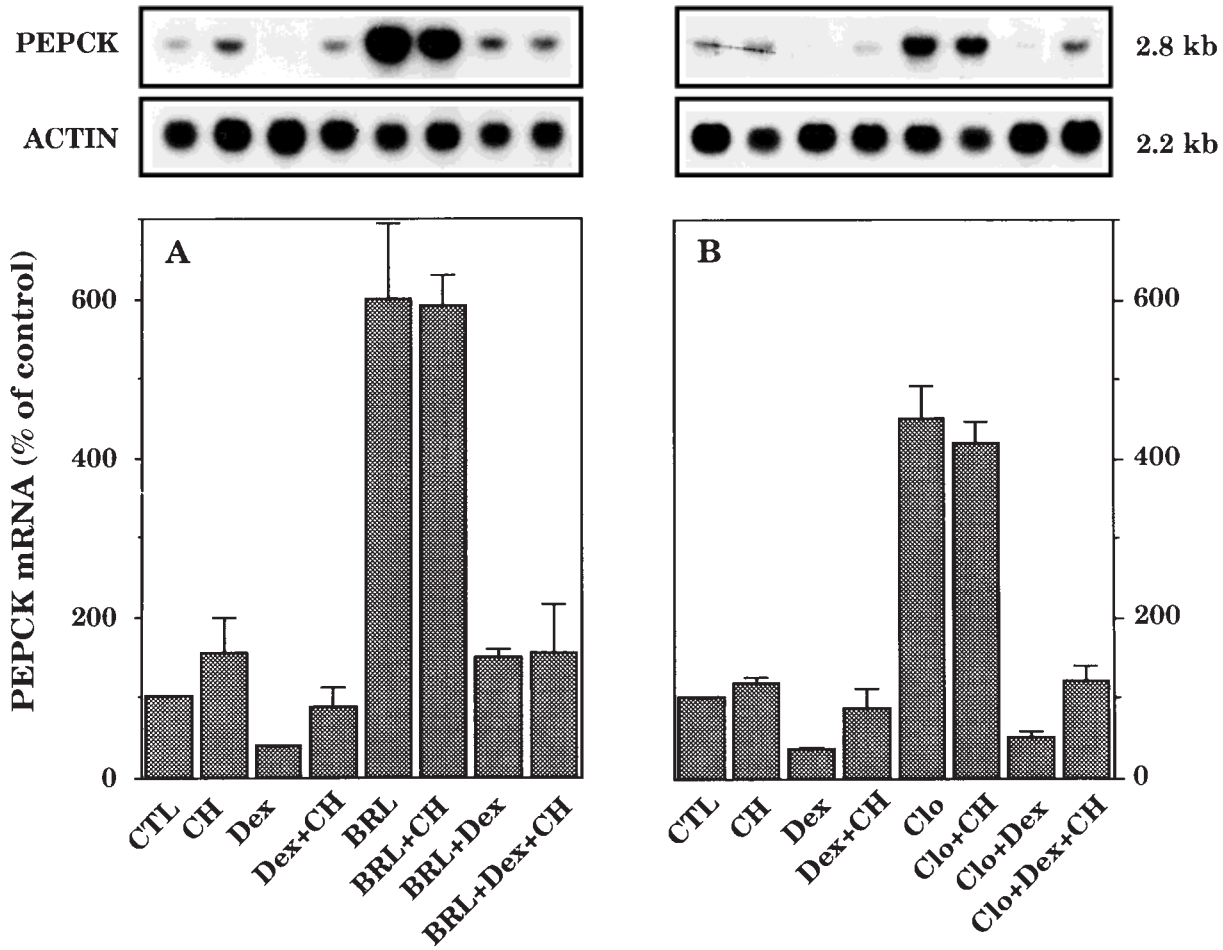

Fig. 5. Effect of cycloheximide on the modulation by BRL 49653, clofibrate, or dexamethasone of PEPCK mRNA in 3T3-F442A adipocytes. 3T3-F442A adipocytes were treated for 4 h with 10 μM cycloheximide (CH) **(A,B),** 1 μM BRL 49653 (BRL) **(A),** 1 mM clofibrate (Clo) **(B)** in the presence or absence of 0.01 μM dexamethasone (Dex) before RNA extraction and analysis. **Top:** Results of typical autoradiograms obtained with blots hybridized sequentially with the PEPCK and actin probes. **Bottom:** Data obtained by scanning densitometry, was normalised for differences in RNA loading by using the actin mRNA signal and expressed as the percent of the PEPCK signal from control (CTL), untreated cells. Each value is the mean of data collected from two independent experiments with duplicate dishes.

PPAR isoform, which may relay clofibrate action, as this fibrate presents no strict specificity.

Unsaturated fatty acids also induce PEPCK mRNA and could do so by PPAR activation. However, stimulation of PEPCK mRNA by thiazolidinediones or fibrates on the one hand, and by fatty acids on the other, appear to present different characteristics. BRL 49653 and clofibrate still strongly stimulate PEPCK mRNA at 24 h [Forest et al., 1997] (Fig. 2), whereas oleate effect is not maintained on a long-term basis [Antras-Ferry et al., 1995], perhaps because of increasing intracellular fatty acid degradation over time. Oleate effect is observed only in glucose-deprived medium [Antras-Ferry et al., 1994b], while clofibrate induces PEPCK mRNA in a similar fashion, whether glucose is present [Antras-Ferry et al., 1995; Forest et al., 1997] or not (this study). BRL 49653 also acts similarly both with glucose (not shown) and without glucose (this study). The observation that fatty acid and fibrate action on gene expression could be different is not unprecedented. Indeed, recent results from Ren et al. [1996] have presented evidence against PPARα as the mediator of polyunsaturated fatty acid (PUFA) regulation of S14 gene transcription in hepatocytes. For that gene, the promoter elements responsible for fibrate and PUFA action are functionally and spatially distinct. For the PEPCK gene, a couple of DR1 elements (RARE1 and PCK2) that bind a heterodimer of PPARγ/

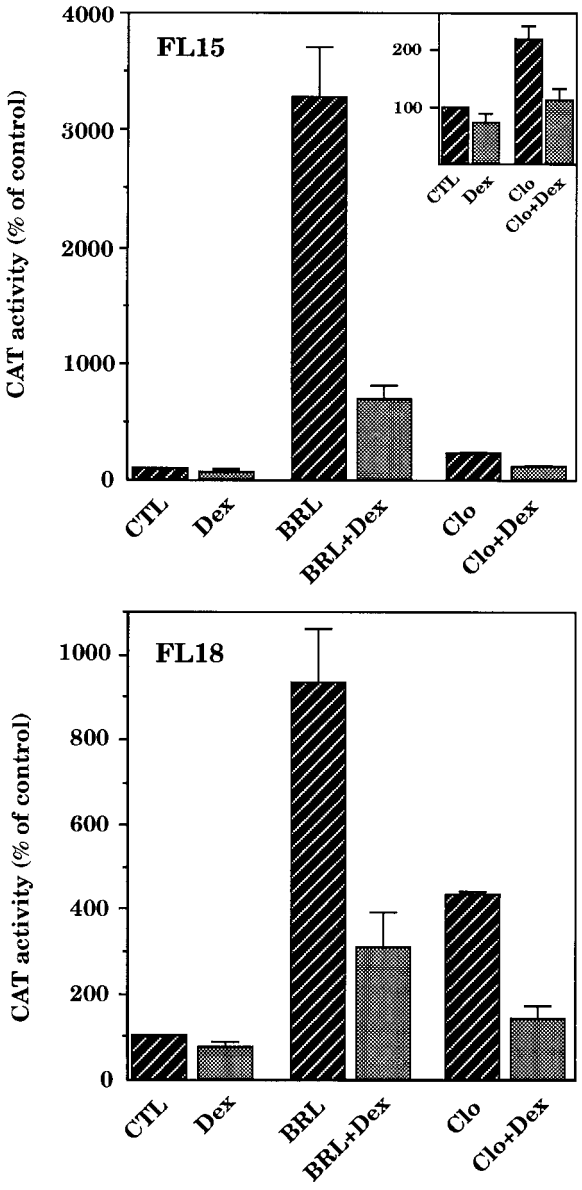

Fig. 6. Effect of BRL 49653, clofibrate, and dexamethasone on CAT activity in FL15 and FL18 PEPCK-CATstable transfectants. FL15 and FL18 adipocytes were maintained for 15 h in Medium 1 (see Materials and Methods) containing 1 μM BRL 49653 (BRL) or 1 mM clofibrate (Clo) in the presence or absence of 0.1 μM dexamethasone (Dex) before measurement of CAT activity. Data are expressed as the percentage of CAT activity from control (CTL), untreated cells. Each value is the mean ±SEM of data collected from three independent experiments with duplicate dishes.

RXRα are at first glance potentially involved in thiazolidinedione, fibrate, and fatty acid induction. However, using transient transfections of 3T3-F442A adipocytes, we have shown that a mutation in RARE1 that prevents protein binding in a gel-shift assay does not impair clofibrate stimulation of the RARE1-mutated −2100 bp PEPCK promoter [Franckhauser-Vogel et al., 1997b]. The implications of RARE1 in thiazolidinedione or of a fatty acid effect, and of PCK2 in overall stimulation, are still to be determined.

In addition to repressing β-agonist or retinoic acid stimulation of PEPCK gene expression in adipocytes [Franckhauser et al., 1994, 1995], glucocorticoids also prevent induction of PEPCK mRNA by thiazolidinediones, fibrates, and fatty acids (Fig. 3). Dexamethasone action is direct (Fig. 5), maximum at 10 nM, a concentration close to the Kd for the glucocorticoid receptor [Hainque et al., 1987] (Figs. 3–5), mediated by the 5′-flanking region of the gene (Fig. 6), and involves the glucocorticoid receptor (Fig. 4). It is well known that thiazolidinedione derivatives such as BRL 49653 are hypolipidemic and decrease insulin resistance, while glucocorticoids increase insulin resistance, a pathological condition linked to an increased concentration of circulating fatty acids. One possible mechanism to explain these opposite effects could be that BRL 49653 and glucocorticoids, respectively, activate and repress adipose tissue glyceroneogenesis, leading, respectively, to a limitation and an enhancement of fatty acid output through their action on PEPCK. If so, dysregulation of adipose PEPCK would play a central role in insulin resistance.

Hence, glucocorticoids exert a dominant inhibitory action on PEPCK gene transcription in adipocytes. This situation is reminiscent to the well-established inhibitory effect of insulin on this same gene in hepatic cells [Granner and Andreone, 1985; Sasaki et al., 1984]. In the latter cells, glucocorticoids enhance PEPCK gene transcription through a complex glucocorticoid response unit (GRU). The GRU encompasses two glucocorticoid receptor binding sites (GREs) located at around −400 bp of the promoter, two elements upstream of the GREs (the RARE1 sequence and an element named AF2) and an element downstream of the GRE, known as RARE2 [Scott et al., 1996a]. Both RARE1 and RARE2 bind a retinoic acid receptor (RAR)/RXR heterodimer and Chicken Ovalbumin Upstream Promoter-Transcription Factor (COUP-TF) [Hall et al., 1995; Scott et al., 1996b]. COUP-TF (and hepatocyte nuclear factor 4 for RARE1) functions as an accessory factor in the hepatic glucocorticoid response [Hall et al., 1995; Scott et al., 1996]. In 3T3-F442A adipo-

cytes, the 2,100-bp PEPCK promoter with a mutation in the RARE1 element retains the induction by clofibrate but specifically lose the glucocorticoid-induced inhibition of the stimulation by clofibrate [Franckhauser-Vogel et al., 1997]. Thus, we have postulated that glucocorticoids could alter the interaction of a protein at the AF1 element, thereby disconnecting communication between the fibrate response element and the basal transcription machinery [Franckhauser-Vogel et al., 1997b]. Another possible explanation could be that cross-coupling exists between PPAR and glucocorticoid receptor signaling through an integrator complex, like the CREB-binding protein (CBP) present in limited amount. Such a mechanism has been proposed in the case of the inhibitory action exerted by the glucocorticoid and retinoic acid receptors on activator protein 1 (AP1; fos–jun) activation [Kamei et al., 1996]. A similar mechanism could also be involved in the dexamethasone repression of the isoprenaline response because CBP also binds to CREB, the postulated factor responsible for cAMP stimulation of PEPCK gene transcription in adipocytes. Whether the AF1 element is also responsible for the antagonistic action of glucocorticoids on thiazolidinedione or fatty acid induction of PEPCK gene transcription is the basis of our future investigations.

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