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Differential induction of stearoyl-CoA desaturase and acyl-CoA oxidase genes by fibrates in HepG2 cells

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Abstract

We studied whether two typical effects of fibrates, induction of stearoyl-CoA desaturase (EC 1.14.99.5) and peroxisome proliferation, are related. The effect of bezafibrate on the activity and mRNA of stearoyl-CoA desaturase and acyl-CoA oxidase in the liver and epididymal white adipose tissue of male Sprague–Dawley rats was determined. The same parameters were measured in HepG2 cells, a cell line resistant to peroxisome proliferation, following incubation with ciprofibrate. Bezafibrate increased the hepatic mRNA levels (14.5-fold on day 7) and activity (9.3-fold on day 15) of acyl-CoA oxidase. Stearoyl-CoA desaturase mRNA levels were transiently increased (2.7-fold on day 7)), while its activity remained increased at the end of the treatment (2.4-fold). In white adipose tissue, bezafibrate increased the mRNA (5-fold) and activity (1.9-fold) of acyl-CoA oxidase, while stearoyl-CoA desaturase was not modified. Ciprofibrate addition to HepG2 cells cultured in 7% fetal bovine serum (FBS) only increased the stearoyl-CoA desaturase mRNA (1.9-fold). When cells were cultured in 0.5% FBS, ciprofibrate increased acyl-CoA oxidase mRNA (2.2-fold), while the increase in stearoyl-CoA desaturase mRNA was identical (1.9-fold). Further, its activity was also increased (1.5-fold). Incubation of HepG2 cells in the presence of cycloheximide did not alter the capacity of ciprofibrate to induce stearoyl-CoA desaturase mRNA, whereas the presence of actinomycin abolished the induction. In addition, preincubation of HepG2 cells with ciprofibrate increased the rate of stearoyl-CoA desaturase mRNA degradation. The results presented in this study suggest that fibrates induce stearoyl-CoA desaturase activity and mRNA levels independently of peroxisome proliferation.

Keywords: Bezafibrate; ciprofibrate; Fibrates, PPAR; Rat liver; Rat white adipose tissue; SCD1; ACO; peroxisome proliferation

1. Introduction

Stearoyl-CoA desaturase (SCD, EC 1.14.99.5) is part of a multienzyme complex that includes cytochrome b_5 and cytochrome b_5 reductase. It is found in the endoplasmic reticulum of mammalian cells. In the presence of O_2 and NADH, this enzyme system catalyses the Δ^9-*cis* desaturation of fatty acyl-CoAs. Its preferential substrates are palmitic and stearic acids, from which it catalyses the synthesis of palmitoleic and oleic acids, respectively [1]. In mouse and rat, SCD activity is displayed by two proteins encoded by the genes *SCD1* and *SCD2*, the expression and regulation of which is tissue-dependent [2]. The hepatic *SCD1* gene, like other lipogenic genes, is induced by hormonal and nutritional factors [3], such as insulin and fat-free or high carbohydrate diets. In contrast, its expression and resulting activity is reduced in diabetes and starvation and in animals feeding on a diet rich in PUFA [3]. The induction of SCD1 activity is preceded by an increase in its mRNA due to a transcriptional activation [4]. Posttranscriptional regulation of SCD1 mRNA by insulin and fructose has also been reported [5]. Further, SCD1 mRNA and activity in rat and mouse increase following administration of peroxisome proliferators [6–9], a heterogeneous group of substances that includes phthalates, herbicides, and hypolipidemic drugs such as fibrates. Peroxisome proliferators cause proliferation of hepatic peroxisomes and endoplasmic reticu-

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Abbreviations: PPAR, peroxisome proliferator-activated receptor; ACO, acyl-CoA oxidase; RT–PCR, reverse transcription–polymerase chain reaction; SCD, stearoyl-CoA desaturase; PUFA, polyunsaturated fatty acid; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; APRT, adenosyl phosphoribosyl transferase; and CIP, ciprofibrate.

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lum, liver hyperplasia, hepatomegaly, and hepatocarcinogenesis in rat and mice [10], but not in other species such as humans or guinea pigs that seem to be resistant to this phenomenon [11,12].

In rodents, induction of liver SCD1 activity and mRNA by fibrates is associated with the induction of the peroxisomal acyl-CoA oxidase (*ACO*) gene [7,8], which encodes the rate-limiting enzyme in the peroxisomal β-oxidation system. This is a widely accepted marker of peroxisome proliferation [13]. Changes in hepatic SCD1 activity in rats treated with peroxisome proliferators are reflected in the ratios of 16:1 to 16:0 and 18:1 to 18:0 fatty acids in hepatic phospholipids [14,15] and liver-derived glycerolipids found in plasma lipoproteins [16]. Furthermore, a peroxisome proliferator response element (PPRE) has been identified within the *SCD1* promoter [9]. Fibrates would thus be expected to induce *SCD1* only in peroxisome proliferator-responsive species.

As mentioned above, humans are generally considered to be non-responsive to peroxisome proliferation. Nevertheless, hyperlipidemic patients treated with fibrates show an increase in the palmitoleic and oleic acid content of their cholesteryl ester, triglyceride, and phospholipid plasma fractions [17–19]. This suggests that administration of fibrates to humans induces SCD.

These results prompted us to explore whether the two phenomena elicited by fibrates, i.e. the induction of the acyl-CoA oxidase enzyme, as a marker of the proliferation of peroxisomes, and the induction of stearoyl-CoA desaturase, are related. We analysed the levels of mRNA and activity of stearoyl-CoA desaturase and acyl-CoA oxidase in liver and epididymal white adipose tissue from rats treated with bezafibrate as well as in HepG2 cells, a hepatoma cell line of human origin that is resistant to peroxisome proliferation, following incubation with ciprofibrate. We show for the first time that fibrates induce increases in SCD1 mRNA levels and activity in cells of human origin. This induction occurs independently of peroxisome proliferation, via a mechanism that involves an increase in the transcription of the *SCD1* gene.

2. Materials and methods

2.1. Drugs

Drugs were purchased from Acofarma (bezafibrate) or provided by ICI-Farma Spain (clofibrate), Sanofi Winthrop Research Division (ciprofibrate), and Parke Davis Spain (gemfibrozil). Actinomycin and cycloheximide (Sigma) were dissolved in ethanol: saline solution (2:1 and 1:10, respectively) and were used at 2.5 and 5 μg/mL concentrations.

2.2. Cell culture

HepG2 human hepatoma cells (European Collection of Cell Cultures) were grown in Ham's F-12 medium supplemented with 7% FBS (GIBCO) (complete medium), and 100 U/mL of penicillin and 100 mg/L of streptomycin (Sigma). Cells were seeded at a density of 7.10^5 cells/100-mm diameter plate. Incubation with clofibrate, ciprofibrate, bezafibrate, or gemfibrozil began at least 32 hr after plating. Stock solutions of these compounds were made in DMSO (Sigma). The concentration of DMSO in the culture medium varied between 0.5 and 1%, depending on the concentration of fibrate used; corresponding control plates were supplemented with the same amount of DMSO. In those experiments performed in FBS-deficient medium, culture medium was replaced with Ham's F-12 containing 0.5% FBS, again 32 hr after plating. Cell viability was assessed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [20]. Cell assays involving drug addition were performed at drug concentrations and incubation times that provided a cell viability of at least 90%.

2.3. Animals and treatments

Thirty-two male Sprague-Dawley rats from Letica, weighing around 130 g, were maintained in a 12-hr light–dark cycle under conditions of constant humidity and temperature (22 $\pm$ 2°) and were fed standard Panlab diet for five days prior to the beginning of the studies. The animals were distributed randomly according to their weight into two groups of 16. Each group was fed a control diet or one containing 0.45% w/w bezafibrate. Throughout the study, the weight and daily food intake of the animals was measured. The 16 rats of each group were killed by decapitation (between 8:00 and 9:00 a.m.) in random groups of 4 after 3, 7, 11, and 15 days of treatment. The diets were prepared as described previously [7]. All procedures were conducted in accordance with principles and guidelines established by the University of Barcelona Bioethics Committee as stated in Law 5/1995 (21st July) of the Generalitat de Catalunya. Blood samples were collected in EDTA tubes and plasma was obtained by centrifugation at 3000 *g* for 10 min at 4°. Plasma levels of total cholesterol and triglycerides were determined by the colorimetric kits CHOD-PAD 290319 and GPO-PAP 701882 from Boehringer Mannheim. Liver and epididymal white adipose tissue samples were frozen immediately in liquid nitrogen and stored at $-80°$ for total RNA isolation. The activity of the stearoyl-CoA desaturase in liver samples and the peroxisomal fatty acid β-oxidation in liver and adipose tissues were assayed at the end of the treatment (15 days) as described previously [7].

2.4. HepG2 cell fatty acid analysis

Hepatoma cell lipids from four control and ciprofibrate plates were extracted following the Bligh and Dyer method [21]. Fatty acid analysis was performed as described previously [14,16,22]: organic phases were partitioned by TLC on 0.2-mm thick silica gel 60 (Merck Kieselgel) using a solvent system of petroleum ether/diethylether/acetic acid (90:30:1, v/v/v). The area corresponding to phospholipids was scraped off and fatty acids were transesterified by heating in a solution of sodium methylate 0.25 N (Supelco) at 50° for 10 min. Fatty acid methyl esters were extracted with hexane (3×2 mL) and analysed using a Hewlett-Packard 5890 gas chromatograph equipped with a bonded silica capillary column (30 m, 0.2 mm internal diameter, Supelcowax). The oven, detector, and injector temperatures were 230, 250, and 270°, respectively. Fatty acids were identified by comparing their retention times with those of fatty acid methyl ester standards obtained from Sigma. Results are expressed as molar percentages.

2.5. RNA isolation

Total RNA was isolated using the ULTRASPEC® reagent (Biotecx). RNA was quantitated according to their absorbance at 260 nm. The integrity of the RNAs was assessed by electrophoresis in 1% agarose gels containing 2% formaldehyde, followed by ethidium bromide staining.

2.6. RT–PCR

mRNA levels were determined by reverse transcription coupled to the polymerase chain reaction (RT–PCR). RT reaction (final volume 20 μL) was performed for 1 hr at 37° using 1 μg (human SCD), 1.5 μg (human ACO), or 0.5 μg (ACO and SCD from liver and adipose rat tissue) as the starting total RNA. The reaction also contained 125 ng random hexamers (Promega), 20 units RNAs in (Promega), 200 units of Moloney murine leukemia virus reverse transcriptase (GIBCO BRL) in 75 mM KCl, 3 mM $MgCl_2$, 10 mM dNTPs (Sigma), 50 mM Tris–HCl pH 8.3. The PCR reaction (50 μL) was carried out using an aliquot of 5 μL of RT reaction and 0.5 μg of each of the specific primers, 200 μM dNTPs, 1 unit of Taq DNA polymerase (GIBCO BRL), and 0.25 μCi of [α-^{32}P]dATP (Amersham) in 20 mM Tris–HCl pH 8.5, 2.5 mM $MgCl_2$. Amplification was carried out by 34 (human ACO), 20 (human GAPDH), 25 (human SCD), 21 (rat liver SCD), or 20 (rat liver ACO, rat adipose ACO and SCD) cycles of PCR. Each cycle consisted of sequential denaturation at 90° for 1 min, annealing at 60° (or 58° for rat ACO) for 1 min 15 sec, and elongation at 72° for 1 min 50 sec, followed by a final elongation step at 72° for 5 min. Primers used were: human *aco* 5′-GCCCAGGTGAAGCCTGATGGA-3′ and 5′-GACTGGTGCCTCACAGCGCTG-3′, human *gapdh* 5′-CAGTCCATGCCATCACTGCCA-3′ and 5′-AGGTGGAGGAGTGGGTGTCGC-3′, rat *aco* 5′-ACTATATTTGGCCAATTTTGTG-3′ and 5′-TGTGGCAGTGGTTTCCAAGCC-3′, rat *aprt* 5′ AGCTTCCCGGACTTCCCCATC-3′ and 5′-GACCACTTTCTGCCCCGGTTC-3′, and *scd1* 5′-GCTCATCGCTTGT-GGAGCCAC-3′ and 5′-GGACCCCAGGGAAACCAG-GAT-3′. To avoid non-specific annealing, cDNA and Taq DNA polymerase were separated from primers and dNTPs by using a layer of paraffin (Fluka); in this way, reaction components came into contact only when the paraffin fused (59°). Rat APRT and human GAPDH were used as controls to normalise the results. For each set of samples, both genes, problem and control, were amplified together except for human ACO and SCD. In this case, the same RT reaction product was used for ACO, SCD, and GAPDH gene amplification. Five microliters of each PCR reaction mixture was subjected to electrophoresis in 5% polyacrylamide gel in 1× Tris buffer EDTA · Na_2. Gels were dried, autoradiographed, and quantified by image analysis (Vilbert-Lourmat v. 4.6). Amplification of each gene yielded a unique band of the expected size (human *aco* 161 bp, human *gapdh* 302 bp, rat *aco* 195 bp, rat *aprt* 329 bp, and *scd1* 521 bp).

2.7. Data analysis

Statistical significance was evaluated using either one-way ANOVA, followed by Duncan's multiple comparison test, or Student's *t*-test. Results are presented as means ± SD (*in vivo*) or means ± SEM (*in vitro*).

3. Results

3.1. Effects of bezafibrate treatment on rat liver and epididymal white adipose tissue ACO and SCD1 mRNA levels and related enzyme activities

As expected, bezafibrate administration to rats increased the hepatic mRNA levels of ACO, the rate-limiting enzyme in the peroxisomal β-oxidation system. Maximal mRNA levels were reached on day 7 of treatment (14.5-fold), but ACO mRNA induction was observed by the 3rd day and remained statistically significant throughout the study (data not shown). At the end of the treatment (day 15), peroxisomal β-oxidation activity was increased 9.3-fold (5.2 ± 0.6 vs 48.4 ± 21.4 nmol/min/mg for control and bezafibrate-treated rats, respectively). On the other hand, SCD1 mRNA levels were also induced by bezafibrate treatment, but this increase was transient, significant only on day 7 of bezafibrate administration (2.7-fold, $P = 0.03$). Nevertheless, SCD1 activity was still increased at the end of the treatment (1.34 ± 0.23 vs 3.21 ± 0.40 nmol/min/mg for control and bezafibrate-treated rats, respectively). These changes were accompanied by a strong hypolipidemic effect, with significant reductions in both plasma cholesterol (40%, 106.3 ± 9.9 vs 63.4 ± 13.9 mg/dL for control and bezafibrate-treated

rats, respectively) and triglyceride concentrations (78%, 228.4 ± 51.5 vs 50.5 ± 3.0 mg/dL for control and bezafibrate-treated rats, respectively) at the end of the treatment. Further, as we have shown previously [23], bezafibrate treatment produced a slight increase in ACO mRNA in adipose tissue compared to the changes in liver. However, this induction was significant from day 7 onwards and maximal (5-fold) on day 15 (data not shown). At the end of the treatment, there was a significant 87% increase in the peroxisomal β-oxidation activity (1.37 ± 0.18 vs 2.56 ± 0.61 nmol/min/mg of protein for control and treated animals, respectively). In contrast, SCD1 mRNA levels were not significantly altered by bezafibrate (data not shown), suggesting that fibrate induction of ACO mRNA in rat epididymal white adipose tissue is not related to any effect of bezafibrate on SCD1.

3.2. ACO and SCD1 mRNA levels after fibrate addition to HepG2 hepatoma cells cultured in complete medium (7% FBS)

None of the fibrates assayed, clofibrate (1 mM), gemfibrozil (0.4 mM), bezafibrate (0.4 mM), or ciprofibrate (0.2 mM), had a significant effect on ACO mRNA levels in HepG2 cells, up to 24 hrs of incubation (data not shown). This is consistent with the resistance of this cell line to the induction of peroxisome proliferation [24]. However, a time-dependent induction of SCD1 mRNA levels was observed after the addition of bezafibrate and ciprofibrate (Fig. 1). Maximal induction was detected with ciprofibrate after 9 hrs of incubation, with no significant alteration of mRNA ACO levels (Fig. 2).

3.3. ACO and SCD1 mRNA levels after fibrate addition to HepG2 hepatoma cells cultured in deficient medium (0.5% FBS)

As mentioned above, PUFA reduce SCD1 activity and mRNA both *in vivo* and in cultured cells [3,25,26]. Under our culture conditions, FBS was the sole exogenous source of PUFA. Thus, we reduced the concentration of FBS to 0.5% to minimise experimental variability and increase the SCD1 signal. Under these conditions, the level of SCD1 mRNA in HepG2 cells was 75% higher than in complete medium (data not shown). Nevertheless, the level of SCD1 mRNA was increased by ciprofibrate addition (Fig. 3), at least up to 0.2 mM ciprofibrate. Surprisingly, ciprofibrate elicited a significant induction of ACO mRNA in HepG2 cells under these conditions (Fig. 4).

As seen with rats [4,14,27], the changes in SCD1 mRNA appear to be related to parallel changes in SCD1 activity, as reflected by the modification of the fatty acid molar composition of liver phospholipids. This also seems to be the case for hepatoma cells treated with ciprofibrate, since in HepG2 phospholipid fatty acids we detected a 16% increase in the ratio between molar percentages of products (palmitoleic and oleic acid) and substrates (palmitic and stearic acid) of SCD1 activity, after 14 hr of incubation of HepG2 cells with 0.2 mM CIP in deficient medium (0.88 vs 0.76 for CIP and control cells, respectively). This increase was even greater (52%) when the ratio between total mono-unsaturated fatty acids and the addition of saturated and polyunsaturated fatty acids was calculated (0.32 vs 0.21 for control and CIP-treated cells, respectively). Thus, changes in molar percentages of mono-unsaturated fatty acids showed that fibrate treatment increased stearoyl-CoA desaturase activity in hepatoma cells.

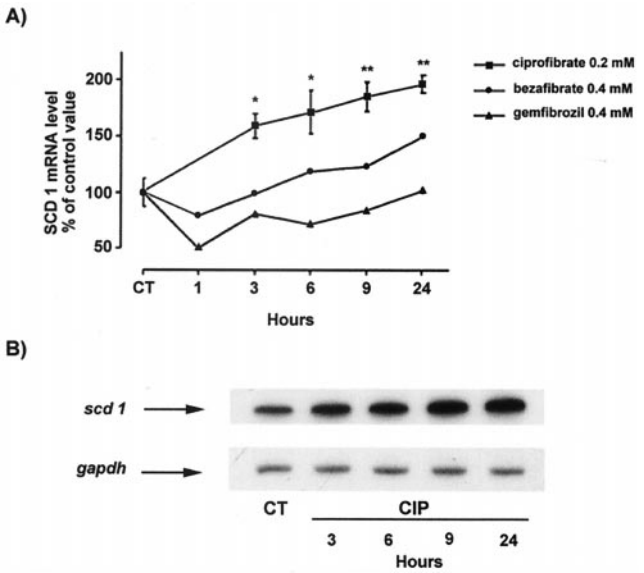

Fig. 1. Time-dependent induction of SCD1 mRNA in HepG2 cells cultured in complete medium after incubation with different fibrates. (a) Percent change in SCD1 mRNA levels in relation to control values (100%) in HepG2 cells after incubation with gemfibrozil 0.4 mM, bezafibrate 0.4 mM, and ciprofibrate (CIP) 0.2 mM at different times. The mRNA values have been normalised using the GAPDH mRNA as a reference. Each point represents the average of the results obtained in two (gemfibrozil and bezafibrate) or three (CIP) different assays. * $P < 0.05$, ** $P < 0.01$ versus control (CT) values. (b) Autoradiography of a representative RT–PCR assay showing the SCD1 signal for CIP-treated and control (CT) cells, with the corresponding signals for the reference GAPDH mRNA.

3.4. Regulation of SCD1 mRNA levels by ciprofibrate in HepG2 cells cultured in deficient medium

To determine the mechanism by which ciprofibrate modifies levels of SCD1 mRNA, we conducted experiments with two well-known inhibitors of the translation and transcription processes, cycloheximide and actinomycin, respectively. Incubation of HepG2 cells in the presence of 5 μg/mL of cycloheximide did not alter the capacity of ciprofibrate to induce SCD1 mRNA (Fig. 5A). However, when parallel experiments were carried out in the presence of 2.5 μg/mL of actinomycin, the effect of ciprofibrate was completely abolished (Fig. 5B). Moreover, in the presence of ciprofibrate plus actinomycin, SCD1 mRNA levels were

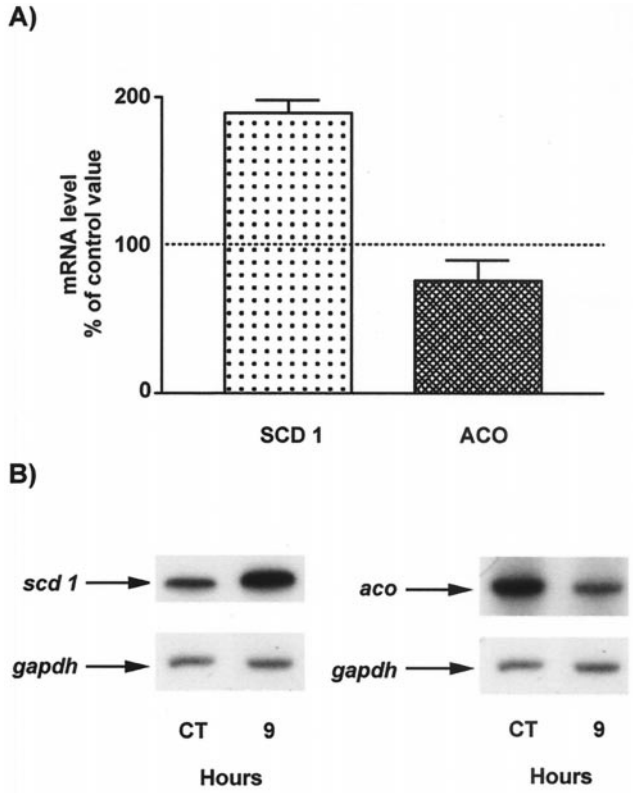

Fig. 2. Differential induction of ACO and SCD1 mRNAs by ciprofibrate addition to HepG2 hepatoma cells cultured in complete medium. (a) Fold induction of ACO and SCD1 levels versus control values (dotted line) after a 9-hr incubation in complete medium with 0.2 mM ciprofibrate. The mRNA values have been normalised using the GAPDH mRNA as a reference. Each bar represents the average of the results obtained in three different assays. (b) Autoradiography of a representative RT–PCR assay showing the ACO and SCD1 signals for ciprofibrate-treated (9 hrs) and control (CT) cells, with the corresponding signals for the reference GAPDH mRNA.

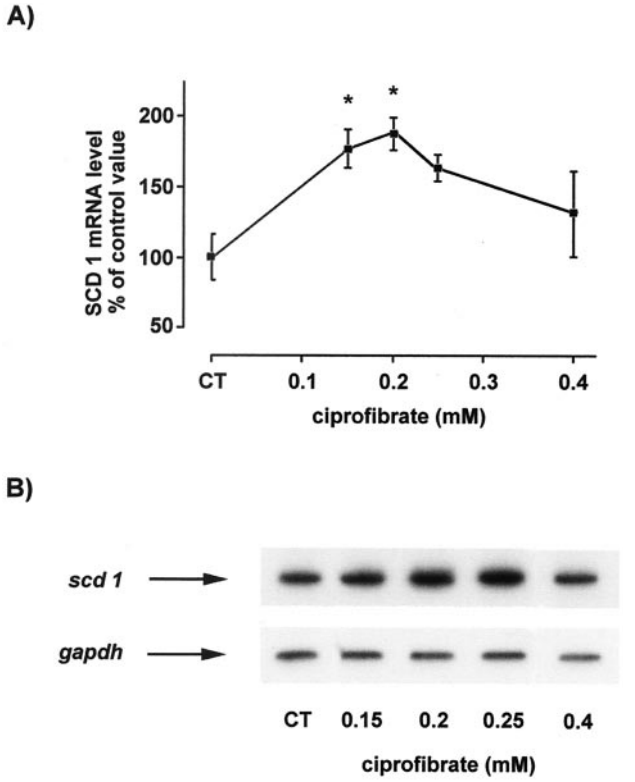

Fig. 3. Concentration-dependent change in SCD1 mRNA levels of HepG2 cells cultured in deficient medium after a 24-hr incubation with ciprofibrate. (a) Percent change in SCD1 mRNA levels in relation to control values (100%) after incubation with different ciprofibrate concentrations. The mRNA values have been normalised using the GAPDH mRNA as a reference. Each point represents the average of the results obtained in three different assays. * $P < 0.05$ versus control (CT) values. (b) Autoradiography of a representative RT–PCR assay showing the SCD1 signals for ciprofibrate-treated and control (CT) cells, with the corresponding signals for the reference GAPDH mRNA.

even lower than corresponding controls incubated solely with actinomycin (Fig. 5B), suggesting that ciprofibrate could also diminish the stability of SCD1 mRNA. Thus, to test this possibility, HepG2 cells were incubated with 0.2 mM CIP for 10 hrs in deficient medium, then changed to fresh medium without ciprofibrate but containing 2.5 μg/mL of actinomycin. The levels of SCD1 mRNA were determined at different incubation times. As seen in Fig. 6, the preincubation with ciprofibrate increased the rate of SCD1 mRNA degradation.

4. Discussion

Here, we show that the induction of stearoyl-CoA desaturase by fibrates is elicited in cultured hepatic cells of human origin, and that this effect is not necessarily linked to peroxisome proliferation produced by these drugs in rats and mice. In this regard, Madsen *et al.* [27] reported that the administration of 1,10-bis(carboxymethylthio)decane acid to rats induced a strong proliferation response with a marginal effect on the expression of the *SCD1* gene. Moreover, we showed that the administration of fibrates to guinea pigs increased the liver phospholipid proportion of mono-unsaturated fatty acids without modifying the activity of the peroxisomal β-oxidation system [22]. Here, we report a different pattern of induction of ACO and SCD1 mRNAs by bezafibrate in rat liver, while in rat adipose tissue bezafibrate induced acyl-CoA oxidase mRNA and activity without modifying the levels of stearoyl-CoA desaturase mRNA, indicating that the two bezafibrate effects were not directly related. In agreement with our results, another study has shown that expression of the *SCD1* gene in 3T3-L1 adipocytes was inhibited by thiazolidindiones, which are PPARγ agonists, while incubation with Wy-14,643, a fibrate-like drug and a strong PPARα agonist, had no effect [28]. Stronger evidence was obtained from the hepatoma HepG2 cell experiments. By changing the culture conditions (using either complete or deficient medium), we have shown that the induction of SCD1 mRNA and activity by fibrates was exclusively dependent on the drug concentra-

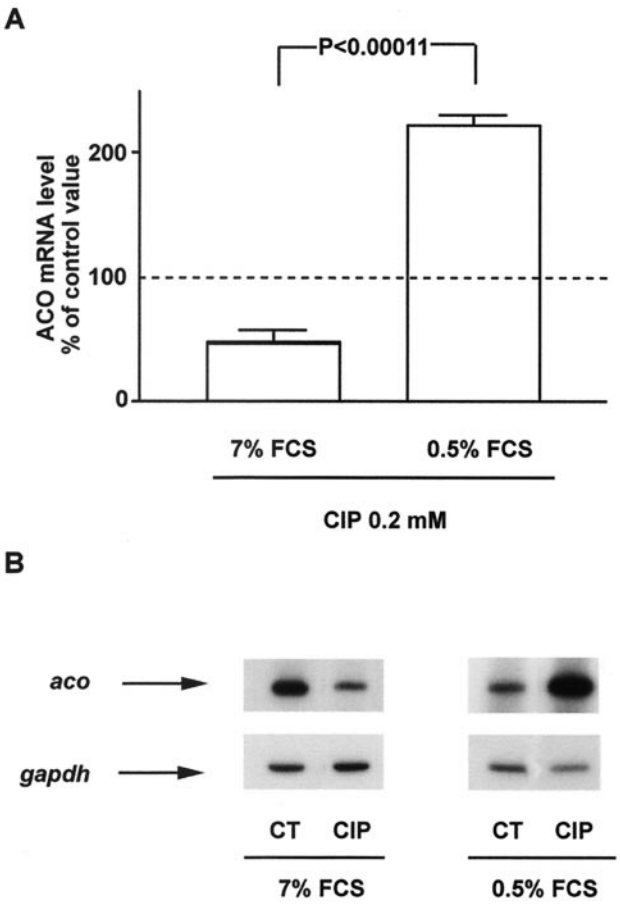

Fig. 4. Change in ACO mRNA levels of HepG2 cells cultured in deficient medium after a 24-hr incubation with ciprofibrate. (a) Percent change in ACO mRNA levels in relation to control values (100%) after incubation with ciprofibrate (CIP 0.2 mM) of cells cultured in complete (7% FBS) or deficient (0.5% FBS) medium. The ACO mRNA values have been normalised using the GAPDH mRNA as a reference. Each point represents the average of the results obtained in three different assays. (b) Autoradiography of a representative RT–PCR assay showing the ACO signals for CIP-treated and control (CT) cells, with the corresponding signals for the reference GAPDH mRNA.

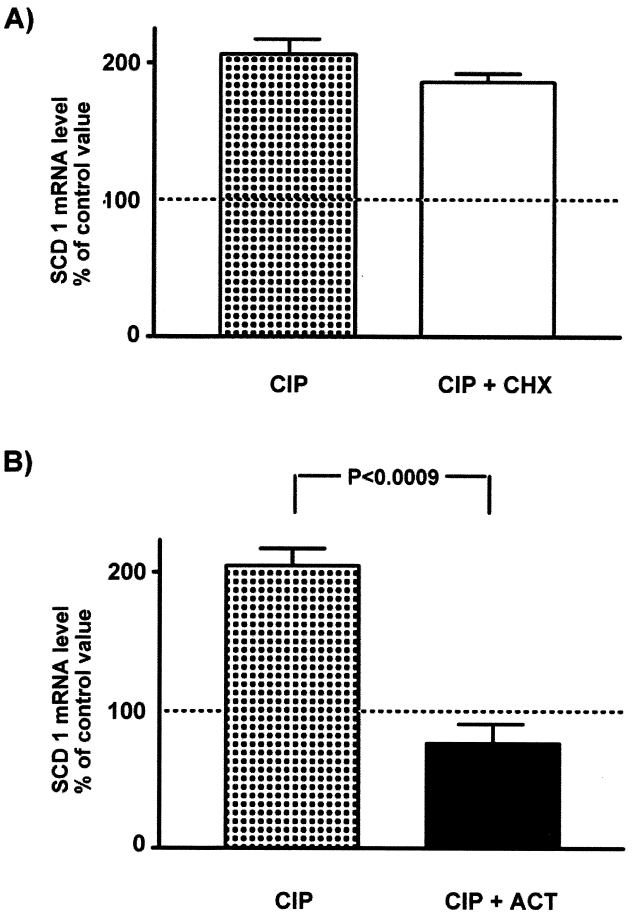

Fig. 5. Modulation by cycloheximide (CHX) and actinomycin (ACT) of ciprofibrate effect on SCD1 mRNA levels of HepG2 hepatoma cells cultured in deficient medium. (a) Percent change in SCD1 mRNA levels with respect to the corresponding control values (with or without CHX, dotted line) after a 9-hr incubation with 0.2 mM ciprofibrate (CIP) in the presence or absence of 5 μg/mL of CHX. The mRNA values have been normalised using the GAPDH mRNA as a reference. Each point represents the average of the results obtained in three different assays. Absolute values were 198 $\pm$ 8, 57 $\pm$ 7, and 109 $\pm$ 15 mRNA arbitrary units for CIP, CHX, and CHX + CIP-treated cells, respectively (mRNA values for untreated cells were fixed at 100). (b) Percent change in SCD1 mRNA levels with respect to the corresponding control values (with or without ACT, dotted line) after a 9-hr incubation with 0.2 mM ciprofibrate (CIP) in the presence or absence of 2.5 μg/mL of ACT. SCD1 mRNA have been normalised against the reference GAPDH mRNA. Each point represents the average of the results obtained in three different assays. Absolute values were 242 $\pm$ 48, 237 $\pm$ 23, and 183 $\pm$ 5 mRNA arbitrary units for CIP, ACT, and ACT + CIP-treated cells, respectively (mRNA values for untreated cells were fixed at 100).

tion used, but was unrelated to the magnitude of the induction of ACO mRNA elicited by the same drug (Figs. 2–4).

Miller and Ntambi [9] identified a peroxisome proliferator response element (PPRE) in the promoter region of the *SCD1* gene between nucleotides −664 and −642, and showed that, in HepG2 cells, clofibrate increased the expression of a construct formed by the SCD1 promoter fused to the chloramphenicol *O*-acetyltransferase (CAT) cDNA when co-transfected with the PPARα receptor. Interestingly, the present results constitute the first report of the same phenomenon in HepG2 cells expressing only their endogenous gene pool, thus establishing the regulation of liver SCD1 by PPARα agonists in liver cells of human origin. In this regard, fibrates do not show any direct effect on liver stearoyl-CoA activity when assayed *in vitro* [29]; they are well-known PPARα agonists [30], and our results show that they control the expression of the *SCD1* gene mainly at the transcriptional level (Fig. 5B), which suggests that they activate a transcription factor. We have also shown a decrease in SCD1 mRNA stability after ciprofibrate addition. It has already been noted that polyunsaturated fatty acids probably act on the unusually long 3′ sequences of SCD1 mRNA, thereby also reducing its stability [31]. As fibrates have been referred to as “fraudulent fatty acids” [32], it is possible that they also act by mimicking the effect of polyunsaturated fatty acids in changing SCD1 mRNA stability.

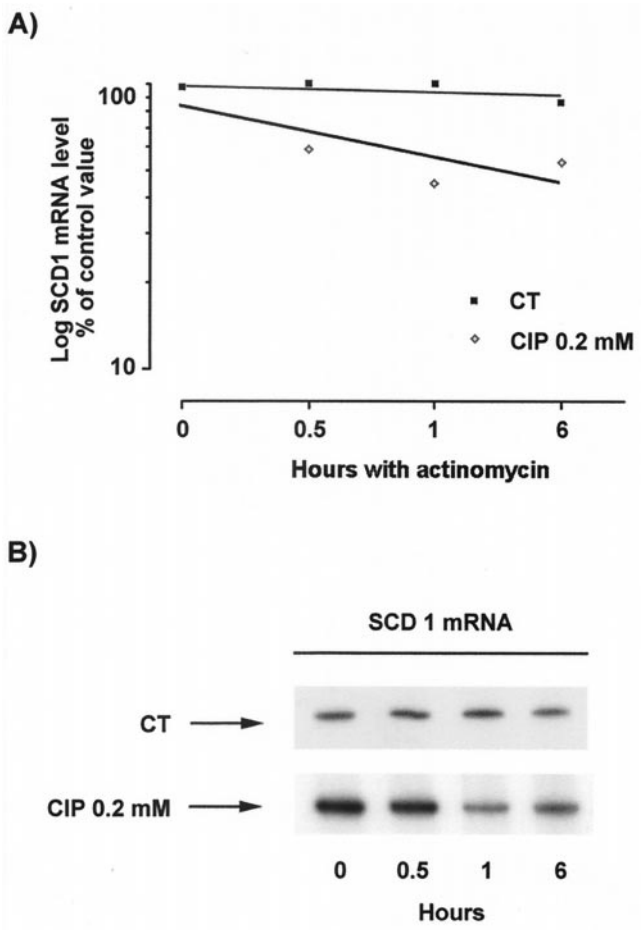

Fig. 6. Time-dependent changes in SCD1 mRNA levels of HepG2 cells cultured in deficient medium plus actinomycin in the presence or absence of ciprofibrate. (a) After 10 hr of incubation with ciprofibrate 0.2 mM (CIP), medium was changed for a fresh one supplemented with 2.5 μg/mL of actinomycin. Cells were harvested at the reported times. Control cells (CT) were manipulated in the same way, but with no addition of CIP. Each point represents the average of the results obtained in two different assays. Experimental points were fitted to lineal equations, $y = (2.9 \times 10^{-4})x + 7.05 \times 10^{3}$, $t½ = 19$ hr 6 min, for CT values, and $y = (-8.6 \times 10^{-4})x + 2.3 \times 10^{3}$, $t½ = 5$ hr 18 min, for CIP values. (b) Autoradiography of a representative RT–PCR assay showing the SCD1 signals for CIP-treated and control (CT) cells.

Thus, the liver *SCD1* gene is regulated by PPARα activation, though by a mechanism that does not involve the induction of peroxisome proliferation-related genes. This implies that the *SCD1* gene could be among the group of genes that code proteins such as apolipoprotein CIII, lipoprotein lipase, or apolipoproteins Apo AI and AII, which are regulated in humans by fibrates through PPARα activation, without a concomitant induction of peroxisome proliferation [30,33–35]. Furthermore, on the basis of our results and those of Kurebayasi *et al.* [28], it can be argued that SCD1 activity, like that of lipoprotein lipase [30], is selectively regulated by PPARα in liver and by PPARγ in white adipose tissue.

We discovered from this study that, by decreasing the amount of FBS added to the culture medium, it is possible to shift HepG2 hepatoma cell response to peroxisome proliferators from resistant to responsive. Our preliminary results suggest this relates to two main factors: a) the relative proportions of the different transcription factors (PPARα, PPARβ, RXR [retinoid x receptor]) related to the process; and b) the reduction in exogenous compounds (i.e. PUFA, hormones) added with the FBS. We are currently working on these findings. It is important to point out that polyunsaturated fatty acids are repressors of stearoyl-CoA desaturase [3,25,26] and furthermore, that they are natural activators of PPARβ [36,37], reported to be a physiological antagonist of PPARα [38].

In summary, the results obtained in the different experimental settings which made up this study show that fibrates, or at least ciprofibrate, are able to induce stearoyl-CoA desaturase mRNA and activity in hepatoma cells of human origin. Although the characteristics of this cell line are not exactly the same as those of human hepatocytes, our results are consistent with other studies showing that fibrate administration to humans is able to increase plasma concentrations of mono-unsaturated fatty acids [17–19]. What is equally important, and also consistent with reported human resistance to peroxisome proliferation [11,12], is the fact that this stearoyl-CoA desaturase induction by fibrates is not directly linked to peroxisome proliferation elicited by these drugs through PPARα activation [30].

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