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Peroxisome proliferator-activated receptor α (PPARα) activators induce hepatic farnesyl diphosphate synthase gene expression in rodents

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Abstract

Fibrates are hypolipidemic drugs that exert multiple effects on lipid metabolism by activating peroxisome proliferator-activated receptor alpha (PPARα) and modulating the expression of many target genes. In order to investigate the link between PPARα and cholesterol synthesis, we analysed the effect of fibrates on expression of the farnesyl diphosphate synthase (FPP synthase) gene, known to be regulated by sterol regulatory element-binding proteins (SREBPs), in conjunction with HMG-CoA reductase. In wild-type mice, both fenofibrate and WY 14,643 induced FPP synthase gene expression, an effect impaired in PPARα-null mice. A three-fold induction was observed in ciprofibrate-treated rat hepatocytes, in primary culture. This effect was decreased in presence of 5,6-dichlorobenzimidazole riboside (DRB) and cycloheximide (CHX), transcription and translation inhibitors, respectively. Acyl-CoA oxidase (AOX), a bona fide PPARα target gene, was induced by ciprofibrate but slower and more strongly than FPP synthase. In addition, induction of FPP synthase gene expression was abolished in the presence of 25-hydroxycholesterol (25-OH Chol). Thus, activation of PPARα by fibrates induced FPP synthase gene expression in both hepatocytes in culture and in mouse liver. This effect is likely to be dependent on cellular sterol level, possibly through SREBP-mediated transcriptional activation.

Keywords: Farnesyl diphosphate synthase; Fibrates; Rat hepatocytes; PPARα-null mice; 25-Hydroxycholesterol

1. Introduction

Fibrates are hypolipidemic drugs that are used in the treatment of hypertriglyceridaemia and combined hyperlipidaemia, and are generally effective in lowering elevated plasma triglycerides and cholesterol [1,2]. These effects are mediated by peroxisome proliferator-activated receptor alpha (PPARα) which is a ligand-activated transcription factor belonging the superfamily of nuclear receptors [3]. Ligands of PPARα are fatty acids and their derivatives and also several pharmacological compounds including fibrates. PPARα controls plasma lipid transport by acting on genes encoding apolipoproteins, lipoprotein lipase, fatty acid transport protein, peroxisomal and mitochondrial fatty acid metabolism enzymes [4–7]. Activated PPARα heterodimerizes with the retinoid X receptor (RXR), itself a receptor for 9-*cis*-retinoic acid, and alters the transcription of target genes after binding to peroxisome proliferator response elements (PPREs) [8,9]. In fibrate-treated rodents, a strong transcriptional activation of genes encoding enzymes of the peroxisomal β-oxidation pathway, is accompanied by peroxisome proliferation and hepatomegaly [10–12]. These effects were completely abolished in PPARα-null mice [13].

Cholesterol synthesis is an important event in the maintenance of hepatic cholesterol homeostasis, which involves also cholesterol uptake, esterification, secretion and degradation to bile acids. The sterol-sensitive transcription

Abbreviations: FPP synthase, farnesyl diphosphate synthase (geranyldiphosphate geranyl*trans*transferase, EC 2.5.1.10); AOX, Acyl-CoA oxidase (EC 1.3.99.2); Ciprofibrate, 2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropionic acid; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; PP, peroxisome proliferator; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; SREBP, sterol regulatory element-binding protein; SRE, sterol response element; CHX, cycloheximide; DRB, 5,6-dichlorobenzimidazole riboside; 25-OH Chol, 25-hydroxycholesterol

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factors, sterol regulatory element-binding proteins (SREBPs) are activators of cholesterogenesis genes [14] including those of HMG-CoA reductase [15] and FPP synthase [16]. SREBPs are membrane-bound transcription factors which are activated by proteolysis [17]. Sterol accumulation in membranes prevents formation of active SREBPs and represses expression of SREBP-dependent enzymes reducing also cholesterol synthesis, whereas sterol depletion produces inverse effects [18]. Among the three SREBP isoforms identified, SREBP-2 is most potent in activating genes of cholesterol biosynthesis [19]. Using gene knockout approaches, it was recently shown that PPARα deficiency was associated with hypercholesterolemia in mice [20]. In addition, the normal regulation of SREBP-dependent genes such as HMG-CoA reductase, was strongly impaired during the diurnal variation of cholesterogenesis [21], and an increased rate of hepatic cholesterogenesis not paralleled by changes in HMG-CoA reductase or SREBP-2 mRNA levels was also reported in PPARα-null mice [22]. Alternatively, the fibrate analog gemfibrozil can stimulate hepatic HMG-CoA reductase activity or cholesterol synthesis in rats [23–25], whereas opposite effects or no effects were observed in response to clofibrate, bezafibrate and WY-14,643 [26–29]. In humans, hepatic HMG-CoA reductase activity was increased in bezafibrate-treated patients suffering from gallstones [30] whereas bezafibrate or gemfibrozil appeared to inhibit cholesterol synthesis or HMG-CoA reductase activity in mononuclear cells [31,32]. Hence, there is some confusion in this area with positive or negative effects of PPARα ligands on HMG-CoA reductase activity and cholesterogenesis, probably depending on species and fibrate treatment conditions.

In order to shed some light on the link between the activity of PPARα and cholesterol synthesis, we have examined the effects of fibrates on FPP synthase gene expression in the liver of both wild-type and PPARα (−/−) mice, and in cultured rat hepatocytes. We show that fibrates induced FPP synthase gene expression, probably indirectly via PPARα, an effect that can be antagonized by oxysterols.

2. Materials and methods

2.1. Animals

Control male C57BL/6 (IFFA-CREDO) and PPARα-null male mice [13] (8 weeks old) were housed under 12 h/12 h dark/light cycles and received a regular chow diet, 3200 Kcal/kg containing 4.3% lipids (UAR). Water and food were available ad libitum. Mice were treated by gavage (200 μl) with fenofibrate (100 mg/kg body weight per day) dispersed in water containing 3% (w/v) arabic gum as vehicle for 14 days. Control mice received the vehicle alone by gavage (3% arabic gum). After intraperitoneal injection of pentobarbital as an anaesthetic, livers were rapidly sampled, frozen in liquid nitrogen and kept at −80 °C until RNA extraction. In addition, control C57BL/6 male mice and PPARα-null male mice were treated by gavage with WY-14,643 (30 mg/kg body weight per day) dispersed in water containing 3% (w/v) arabic gum for 8 days.

2.2. Hepatocyte preparation

Hepatocytes were isolated and cultured as previously described [33] and modified [34]. Briefly, hepatocytes were prepared by “in situ” collagenase perfusion from Wistar male rats (180–200 g). Isolated hepatocytes were seeded in 25 cm^2 flasks at 2×10^6 cells/flask in Leibovitz’s L-15 medium, containing 10^{-6} M insulin and hydrocortisone, 40 μg/ml streptomycin and 40 IU/ml penicillin G and supplemented with 10% delipidated fetal calf serum (Sigma), a serum containing low concentrations of cholesterol (60 mg/l) and triglycerides (150 mg/l). Cell viability was higher than 97% as judged by Trypan blue exclusion. The medium was replaced 4 h after seeding.

2.3. Treatment of hepatocytes

Ciprofibrate treatment: 18 h after seeding, the medium was replaced by Leibovitz’s L-15 medium containing 100 μM, 250 μM or 500 μM ciprofibrate. Ciprofibrate was added in DMSO (0.1% final concentration). Treatment was carried out for 8, 12, 24, 48 and 72 h and culture media were replaced after 24 and 48 h of incubation. Controls also received 0.1% DMSO. The absence of cytotoxicity was controlled by measuring lactate dehydrogenase activity in the medium after treatment.

25-OH Chol treatment: hepatocytes were treated with 25-OH Chol at 0.1, 1 and 10 μg/ml concentrations. Control hepatocytes received vehicle alone (ethanol, 0.1% final concentration). No detectable toxicity was observed in control cultures.

Protein and RNA synthesis inhibitors treatment: cells were treated simultaneously with 500 μM ciprofibrate and the protein synthesis inhibitor cycloheximide (CHX), or the RNA synthesis inhibitor 5,6-dichlorobenzimidazole riboside (DRB), at a concentration of 50 μg/ml for 12 h.

2.4. Enzyme assays

After each treatment, cell monolayers were washed with 4 ml PBS, recovered with a rubber policeman and centrifuged twice at $50 \times g$ for 10 min. Pellets were resuspended in 500 μl of 5 mM MOPS buffer (pH 7.4) containing 0.25 M sucrose, 1 mM EDTA and 0.1% ethanol. Cell homogenates were obtained by sonication (3×10 s) on ice.

FPP synthase activity was determined by the conversion of [1-^{14}C]isopentenyl diphosphate (IPP, Amersham Biosciences) to [^{14}C]farnesyl diphosphate and other derivative products, according to Krisans et al. [35].

AOX activity, used as a marker of treatment efficacy for PPARα activation and transcriptional regulation [36], was determined by measuring the production of palmitoyl-CoA dependent-H_2O_2 by a fluorometric assay, as previously described [37]. Protein content was determined by the method of Bradford, using BSA as standard.

2.5. RNA preparation and Northern blot analysis

RNA was isolated from control and treated hepatocytes according to Chomczynski and Sacchi [38]. Total RNA (20 μg) was electrophoresed in 1% agarose gels containing 0.6 M formaldehyde, and then transferred to Hybond-N nylon membranes (Amersham) with 1.5 M sodium chloride, 1.5 M sodium citrate (10× NaCl/Cit, pH 7.0) as the transfer buffer. Probes were prepared with [α-^{32}P] dCTP by using a Ready Prime Random Labelling kit (Amersham). A human FPP synthase cDNA fragment of 0.250 kb, a rat AOX cDNA fragment of 1.7 kb, a rat HMG-CoA reductase cDNA fragment of 0.978 kb, a rat phosphoprotein cDNA fragment of 0.443 kb (36B4 probe) and a mouse β-actin cDNA fragment of 1 kb were used for preparing the probes. Hybridisation and washes were performed using standard procedures. Membranes were then exposed to X-ray films (X-OMAT, Kodak) with an intensifying screen. The levels of mRNAs were quantified with a CS-9000 Shimadzu densitometer, and all mRNA expression levels were normalized to the intensity of the β-actin or 36B4 signal.

3. Results

3.1. FPP synthase gene expression is induced by fenofibrate and WY-14,643 in mouse liver via PPARα

Treatment of wild-type mice with fenofibrate resulted in a 2.2-fold FPP synthase mRNA increase (Fig. 1). In PPARα (−/−) mice treated with fenofibrate, a small apparent increase in the amount of mRNA was observed, but it was below the limit of statistical significance. In comparison, fenofibrate treatment of wild-type mice induced AOX gene expression by six-fold, whereas no induction was observed in PPARα (−/−) mice after treatment (data not shown). These results indicate that PPARα activation by fenofibrate contributes significantly to FPP synthase gene induction, although some other factors might be involved in the modest residual induction observed in PPARα (−/−) mice.

In addition, treatment of mice with WY-14,643, a PPARα agonist more selective than fenofibrate [39], resulted in a 2.1-fold increase of FPP synthase mRNA (Fig. 1). By contrast, this up-regulation was fully impaired in PPARα (−/−)-treated animals. This result suggests that fenofibrate might also activate, albeit modestly, other PPAR isoforms, which may not be the case for WY-14,643.

3.2. PPARα activators induce FPP synthase gene expression in rat hepatocytes

Rat hepatocytes were treated with 500 μM ciprofibrate for 8–48 h periods, in culture medium without serum. FPP

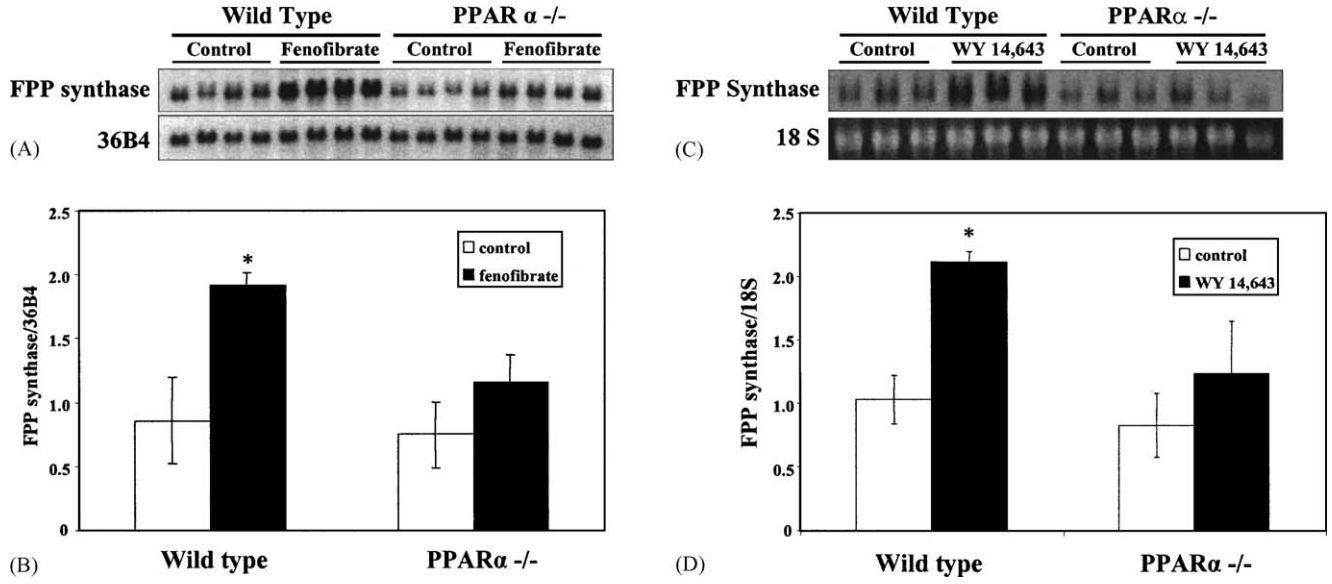

Fig. 1. Effect of fenofibrate and WY 14,643 treatments on FPP synthase gene expression in wild-type and PPAR (−/−) mice. Mice were fed control chow diet or were treated by gavage with fenofibrate (100 mg/kg body weight per day), or with WY 14,643 (30 mg/kg body weight per day). (A) Northern blot analysis of hepatic FPP synthase mRNA, and 36B4 mRNA used as control, after fenofibrate treatment. (B) Levels of FPP synthase mRNA after normalization to 36B4, after fenofibrate treatment. (C) Northern blot analysis of hepatic FPP synthase mRNA, and ethidium bromide staining of the membrane used as control, after WY 14,643 treatment. (D) Levels of FPP synthase mRNA after normalization to 185 RNA, after WY 14,643 treatment. Statistically different from control wild-type animals ($P < 0.001$, Student's t-test).

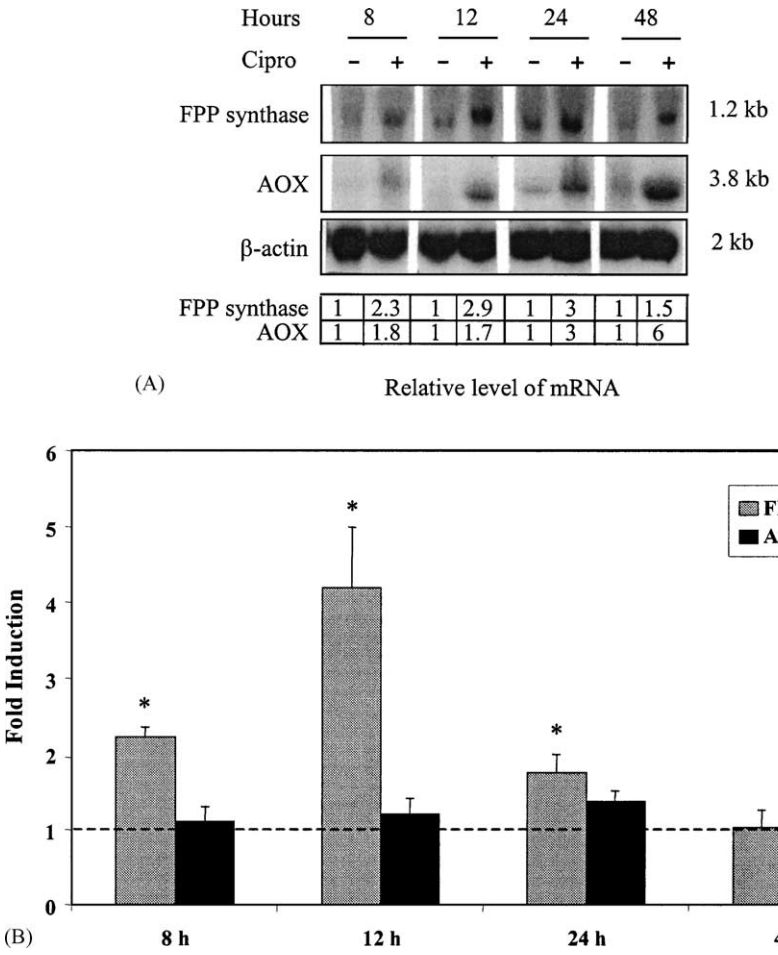

Fig. 2. Effect of ciprofibrate on FPP synthase and AOX gene expression in cultured rat hepatocytes. Hepatocytes were incubated with 500 μM ciprofibrate (cipro) for up to 48 h. (A) Northern blot analysis of FPP synthase and AOX mRNAs. The table shows the mRNA levels expressed relative to the mRNA contents in control, untreated cells after normalization to the level of β-actin mRNA. (B) FPP synthase and AOX activity values expressed relative to activities in control cells, for each time point. FPP synthase activity value in control cells was 153 ± 35 nmol/h/mg protein after 12 h of culture. AOX activity value in control cells was 120 ± 7 nmol/h/mg protein after 12 h of culture. Values are means ± S.D. ($n = 6$). Statistically different from control ($P < 0.05$) (Student's *t*-test).

synthase mRNA level increased significantly to 2.3-fold above control after 8 h of treatment, rising to a maximum of three-fold after 12 and 24 h (Fig. 2A); this was paralleled by a comparable increase in enzyme activity (Fig. 2B). Induction of mRNA level remained after 48 h, albeit at a lower (1.5-fold) level, while the enzyme activity had returned to basal values. In addition, AOX mRNA level and activity were analysed as a positive control of PPARα-dependent gene expression. The AOX mRNA level increased gradually with the ciprofibrate incubation time, reaching a maximum of six-fold after 48 h of treatment. Enzyme activity showed no significant change in the first 24 h, but increased to 2.6-fold after 48 h.

In order to determine whether ciprofibrate induces increased FPP synthase gene expression and/or mRNA stabilisation, hepatocytes were treated with the transcription inhibitor 5,6-dichlorobenzimidazole riboside, with or without ciprofibrate. No induction of either FPP synthase mRNA or enzyme activity was obtained in response to ciprofibrate in the presence of DRB, whereas the fibrate induced the mRNA and enzyme activity levels by 3.4- and 4.2-fold, respectively, suggesting that ciprofibrate increased transcription (data not shown). In order to analyse the requirement for translation, hepatocytes were treated with cycloheximide, with or without ciprofibrate. No more FPP synthase mRNA induction by ciprofibrate was observed in the presence of CHX, suggesting at least a partial need for protein synthesis for the ciprofibrate effect (data not shown). Similar antagonizing effects of CHX were observed for AOX, as previously reported [40].

3.3. Differential effects of ciprofibrate on FPP synthase and AOX activity kinetics

For the concentration of 500 μM ciprofibrate during 8–48 h, FPP synthase and AOX gene expression was induced in a time-dependent manner (Fig. 2). Complementary kinetic studies were performed with 100 and 250 μM

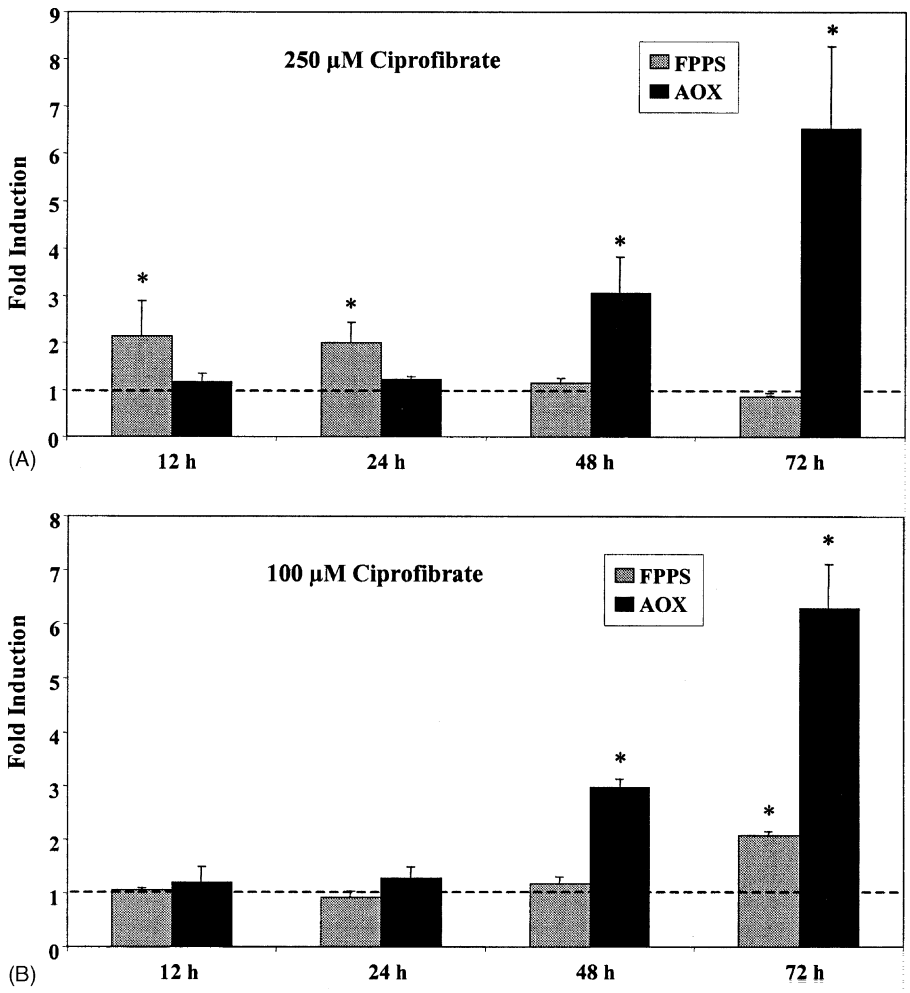

Fig. 3. Effect of ciprofibrate on FPP synthase and AOX activity values in cultured rat hepatocytes. (A) Treatment with 250 μM ciprofibrate and (B) Treatment with 100 μM ciprofibrate. Values are means ± S.D. ($n = 6$). Statistically different from control ($P < 0.05$) (Student's t-test).

ciprofibrate for 12–72 h periods (Fig. 3). The increase in FPP synthase activity was faster and larger as the concentration of ciprofibrate increased. A two-fold increase in enzyme activity was observed for 100 μM ciprofibrate after 72 h and a similar two-fold increase was observed after 12–24 h for the dose of 250 μM (Fig. 3A). The strongest induction (4.3-fold) was found after only 12 h of treatment with 500 μM, the induction after 8 h being 2.2-fold (Fig. 2B). FPP synthase activity next decreased to values similar to those of the controls. In contrast, AOX activity induction kinetics were comparable for 100, 250 and 500 μM ciprofibrate (Fig. 2B and 3B). The enzyme activity was significantly induced after 48 h of treatment at each ciprofibrate concentration, and maximum induction factors of 6.3- and 6.6-fo1d were observed after 72 h of treatment with 100 and 250 μM ciprofibrate, respectively. Therefore, ciprofibrate induced expression of both FFP synthase and AOX genes, with increased mRNA levels and enzyme activities. However, the induction kinetic of FPP synthase activity was somewhat faster, but with a lower magnitude than that of AOX.

3.4. The effect of fibrates is dependent on cellular sterol levels

Since FPP synthase is well known to be regulated by the cellular sterol content through a feedback regulatory mechanism [16], we investigated the effect of reduction in exogenous lipids (i.e. cholesterol) supply on fibrate-mediated up-regulation of FPP synthase. In our model of hepatocyte culture, the unique source of cholesterol is the serum, as mentioned by Rodriguez et al. [41]. Cultured rat hepatocytes were treated with 250 μM ciprofibrate for 24 h periods in medium without serum provoking low cellular cholesterol level, or with delipidated bovine calf serum provoking higher cellular sterol level. The ciprofibrate-induced increase in FPP synthase mRNA level was observed in the absence of serum, whereas delipidated serum suppressed all induction (Fig. 4), suggesting that the effect of fibrates was dependent on cellular sterol content. Similarly, the mRNA level of HMG-CoA reductase, another cholesterogenesis gene regulated by cellular sterol levels, was increased by ciprofibrate treatment in absence of serum (Fig. 4).

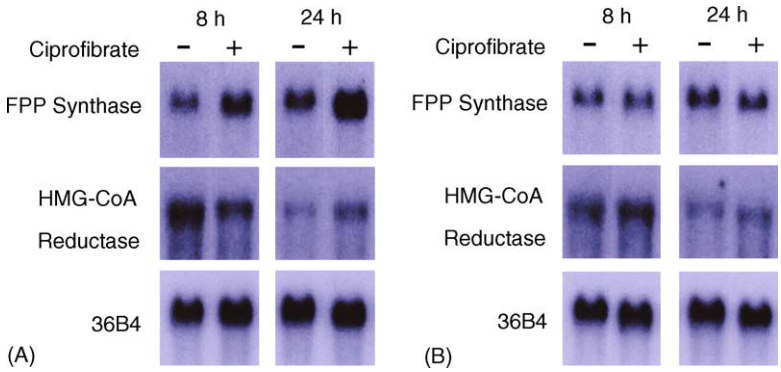

Fig. 4. Effect of ciprofibrate on FPP synthase and HMG-CoA reductase expression in cultured rat hepatocytes. (A) Absence of serum. (B) Presence of delipidated bovine calf serum. Northern blot analysis of FPP synthase and HMG-CoA reductase mRNAs, after a 250 μM treatment for 8 and 24 h. 36B4 mRNA was used as control.

Next, we investigated the combined effects of ciprofibrate and 25-OH Chol, a potent regulator preventing SREBP cleavage and repressing SREBP-dependent gene expression [18]. Cultured hepatocytes were treated for 12 h with 500 μM ciprofibrate and 25-OH Chol at concentrations of 0.1 μg/ml, 1 μg/ml or 10 μg/ml, sterol concentrations expected to suppress FPP synthase expression. Indeed, FPP synthase activity decreased by about two-fold with 10 μg/ml 25-OH Chol (Fig. 5A). In addition, FPP synthase was increased by ciprofibrate (4.2-fold) and this increase was prevented by 25-OH Chol, an effect which was complete for the two lowest doses and partial for the highest dose (Fig. 5A). These results show that 25-OH Chol can fully antagonise ciprofibrate-mediated induction (0.1 and 1 μg/ml), although this may be less apparent for the highest dose (10 μg/ml) since, in this case a partial suppression of basal enzyme activity was also noted. Under the same treatment conditions, ciprofibrate increased FPP synthase mRNA level (2.9-fold), and this increase was much less significant (1.2- and 1.3-fo1d) when hepatocytes were also treated by 25-OH Chol at 1 and 10 μg/ml, respectively (Fig. 5B). Hence, 25-OH Chol antagonised the positive effect of ciprofibrate on FPP synthase gene expression.

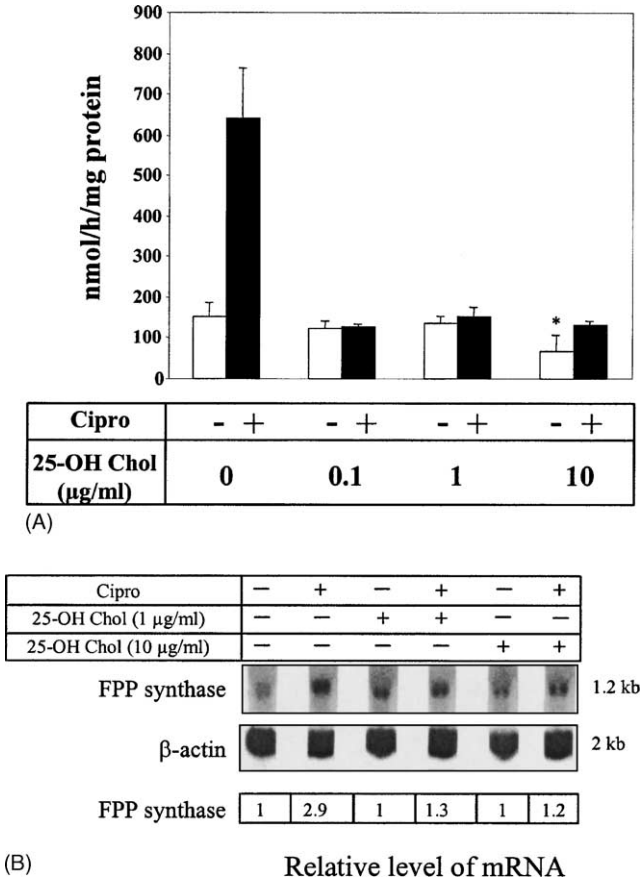

Fig. 5. Effect of 25-OH Chol on ciprofibrate-induced FPP synthase activity (A) and mRNA level (B) in cultured rat hepatocytes. Rat hepatocytes were treated with 500 μM ciprofibrate (Cipro) in the absence or presence of increasing doses of 25-OH Chol for 12 h. FPP synthase activity values are means ± S.D. ($n = 6$). Statistically different from untreated hepatocytes ($P < 0.05$) (Student's t-test). FPP synthase mRNA levels were normalized to the level of β-actin mRNA used as a control.

4. Discussion

The aim of the present study was to obtain a better understanding of the link between PPARα and cholesterol synthesis, precisely of the effects of PPARα agonists on FPP synthase gene expression. The main finding is that fibrates induce FPP synthase gene expression in the liver of wild-type mice, and in cultured rat hepatocytes, whereas only little induction could be obtained in PPARα (−/−) mice, indicating that the inducing effect of fibrate depends largely on the activation of PPARα. A strong argument supporting an active role of PPARα in up-regulation of FPP synthase was provided by the induction of FPP synthase expression in mice treated with the selective PPARα-agonist WY-14,643, and the absence of a similar effect in PPARα (−/−) mice. The mechanisms by which fenofibrate exerts a positive role on FPP synthase expression in mice could also marginally involve the PPARγ receptor isoform. Indeed, fenofibrate has been suggested to weakly activate PPARγ [39], and to up-regulate expression of HMG-CoA

reductase in THP-1 macrophages [42]. However no induction of FPP synthase expression was observed following treatment of PPARα (−/−) mice with rosiglitazone, a PPARγ selective activator (Chevillard, personal communication). Fibrate-induced FPP synthase gene expression has also been observed in the liver of mice treated with 2-diethylhexylphalate (DEHP), another peroxisome proliferator [43]. In addition, it was shown to be correlated with increased HMG-CoA reductase activity or cholesterol synthesis in liver of gemfibrozil-treated rats [23–25] and of bezafibrate-treated patients [30]. The opposite effects reported in the same species, as discussed earlier, stem probably from differences between experimental approaches in vivo and in vitro, particularly the dissimilarities in the potency of fibrates, drug dosages and treatment lengths.

PPARα is highly expressed in liver and transactivates genes principally involved in lipid oxidation and lipoprotein metabolism [6,7]. There have been no reports to indicate that the promoter regions of the cholesterogenic genes contain PPAR response elements. However sequence analysis of 770 bp from the proximal promoter region of the rat FPP synthase gene [44] showed a DNA motif AGGTCA, at position −504 to −509, resembling a half PPRE sequence. We evaluated the transcriptional activity of PPARα on this promoter by transient transfection assays. Co-transfection experiments in rat hepatoma cells of a PPARα expression plasmid and the FPP synthase promoter did not show any induction in reporter gene expression, suggesting that this half-site is not functional (data not shown). Further investigations will be required to determine whether functional PPRE sequences can be found elsewhere within the 5′-flanking region of the gene.

Alternatively, a variety of genes are not apparently direct targets for PPARα, and changes in transcription may be secondary to the regulation of PPARα target genes in compensatory response to metabolic alterations caused by PPARα agonists. A plausible explanation for the up-regulation of FFP synthase is that fibrates could trigger a cholesterol requirement in hepatocytes and also increase the active SREBP level. This could arise since, in rodents, fibrates strongly induce peroxisome proliferation resulting in cellular hyperplasia and hypertrophy, and liver enlargement [11,12]. It is conceivable that induction of FPP synthase may be correlated with fibrate-mediated peroxisome proliferation in cultured hepatocytes, a phenomenon that also involves a massive increase in BFE (bifunctional enzyme) protein levels [45]. Additionally, the depletion of cholesterol in liver could result from the fibrate-mediated increase of biliary cholesterol secretion. In ciprofibrate-treated mice, biliary cholesterol secretion which is coupled to that of phospholipids controlled by Mdr2 P-glycoprotein, may be increased in correlation with induced Mdr2 P-glycoprotein expression [46]. In these animals, expression of ABC genes, ABCA1, ABCG5 and ABCG8, encoding transporters which were suggested to play a role in cholesterol secretion, did not change significantly. In patients with different types of hyperlipoproteinemia, the treatment with fibrates increases biliary cholesterol secretion and cholesterol saturation of bile [47]. Thus, fibrates may cause a state of cellular cholesterol deprivation by at least two different mechanisms, resulting in increased amounts of active SREBPs and enhanced transcription of FPP synthase. In favour of this hypothesis, gemfibrozil stimulated the SREBP-2 transactivation pathway in rats in correlation with induced HMG-CoA reductase gene expression [24]. On the other hand, recent studies have suggested that PPARα may contribute to the regulation of cholesterogenic SREBP-dependent genes depending on the hormonal changes in circadian cycle [21].

25-OH Chol is capable of preventing formation of active SREBP transcription factors and to reduce SREBP-dependent gene expression [18]. This mechanism could suppress the ciprofibrate-induced FPP synthase gene expression in cultured rat hepatocytes. In addition the liver X receptors (LXRs), known to be activated by oxysterols [48], could be involved. Interestingly, the recent analysis of LXRα-null mice demonstrated that several enzymes involved in sterol synthesis, including FPP synthase, were induced compared with levels in control mice, under conditions of low dietary cholesterol, suggesting that LXRα functions as an essential regulatory component [49]. Indeed, LXRα was identified as one PPARα-interacting factor. It inhibited the binding of PPARα/RXRα to PPREs in vitro and antagonised transcriptional activation by PPARα in vivo [50].

In summary, hepatic FPP synthase is up-regulated by fibrate treatment in rat and mouse liver. Impaired up-regulation in PPARα (−/−) animals provides evidence that PPARα activation is involved in this induction. Differential induction kinetic of FPP synthase and AOX gene expression by fibrates and requirement of low cellular sterol content for up-regulation of FPP synthase support the hypothesis that this effect could occur by the SREBP-mediated transcriptional activation. Divergent data reported in a given species, rat or human, on the effect of fibrates on HMG-CoA reductase activity and cholesterol synthesis could reflect an indirect phenomenon related to PPAR activation and disturbances of hepatic cholesterol flux. Such a mechanism could coordinate HMG-CoA reductase gene expression with that of other SREBP-sensitive cholesterogenic genes in response to PPARα activation, and could result in cholesterol synthesis adaptation in order to compensate for the changes in hepatic cholesterol flux provoked by PPARα agonists.

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Paris, France), respectively. PPAR (−/−)mice were originally generated in the laboratory of Dr. F.J. Gonzalez (NIH, Bethesda, MA).

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