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# Suppression of hepatocyte nuclear factor-4α by acyl-CoA thioesters of hypolipidemic peroxisome proliferators

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**Abstract**

Hepatocyte nuclear factor-4α (HNF-4α) modulates the expression of liver-specific genes that control the production (e.g. apolipoprotein [apo] A-I and apo B) and clearance (e.g. apo C-III) of plasma lipoproteins. We reported that the CoA thioesters of amphipathic carboxylic hypolipidemic drugs (e.g. clofibric acid analogues currently used for treating hyperlipidemia in humans and substituted long-chain dicarboxylic acids) were formed *in vivo*, bound to HNF-4α, inhibited its transcriptional activity, and suppressed the expression of HNF-4α-responsive genes. Hypolipidemic PPARα (peroxisome proliferator-activated receptor alpha) activators that were not endogenously thioesterified into their respective acyl-CoAs were shown to be effective in rats but not in humans, implying that the hypolipidemic activity transduced by PPARα in rats was PPARα-independent in humans. The suppressed acyl-CoA synthase of PPARα knockout mice left unresolved the contribution made by the acyl-CoA/HNF-4α pathway to the hypolipidemic effect of PPARα agonists in rodents. Hence, suppression of HNF-4α activity by the CoA thioesters of hypolipidemic "peroxisome proliferators" may account for their hypolipidemic activity independently of PPARα activation by their respective free carboxylates. The hypolipidemic activity of peroxisome proliferators is mediated in rats and humans by the PPARα and HNF-4α pathways, respectively. 

*Keywords:* Lipoproteins; PPARα; Apo C-III; Hypolipidemic drugs; Fibrates; Medica

## 1. Introduction

HNF-4α [1] controls the expression of liver genes coding for apolipoproteins (AI, AII, AIV, B, C-III), coagulation factors (VII, IX, X), transcription factors (HNF-1), and others [2]. Transcriptional activation by HNF-4α is mediated by its binding as homodimer to DR-1 promoter sequences of target genes. Fatty acyl-CoAs longer than C12 specifically bind to the LBD of HNF-4α, resulting in modulating its transactivation or affinity for its cognate responsive enhancer, or in shifting its oligomeric–dimeric equilibrium as a function of the chain length and degree of saturation of these fatty acyl-CoAs [3]. Binding of saturated (C14:0)-CoA or (C16:0)-CoA results in HNF-4α activation, whereas (ω-3) polyunsaturated (C18:3)-CoA, (C20:5)-CoA, or (C22:6)-CoA suppresses its transcriptional activity. Activation of HNF-4α by its agonists may account for the reported increase in blood lipids induced by dietary saturated fatty acids of C12–C16, while suppression of HNF-4α activity by its antagonists may account for the hypolipidemic effect exerted by dietary (ω-3) polyunsaturated fatty acids [4]. Since hypolipidemic PP (e.g. clofibric acid analogues currently used for treating hyperlipidemia in humans M14 (N = 8), M16 (N = 10) or M18 (N = 12) homologues of the MEDICA (M) series (HOOC—$CH_2$—$C(CH_3)_2$—$(CH_2)_n$—$C(CH_3)_2$—$CH_2$—COOH) [5]) are amphipathic carboxylates that may serve as substrates for the fatty acyl-CoA synthase [6], their hypolipidemic effect could be similarly accounted for by binding of their CoA thioesters to HNF-4α and the suppression of its transcriptional activity.

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*Abbreviations:* apo, apolipoprotein; CAT, chloramphenicol acetyltransferase; Cl-DICA, α,α′-tetrachloro-tetradecane dioic acid; ESI, electron spray ionization; GST, glutathione *S*-transferase; HNF-4α, hepatocyte nuclear factor-4alpha; LBD, ligand binding domain; M14, β,β′-tetramethyl-tetradecane dioic acid; M16, β,β′-tetramethyl-hexadecane dioic acid; M18, β,β′-tetramethyl-octadocane dioic acid; MEDICA, β,β′-tetramethyl-dicarboxylic acid; PP, peroxisome proliferators; PPARα, peroxisome proliferator-activated receptor alpha; and VLDL, very low density lipoprotein.

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## 2. Materials and methods

### 2.1. Recombinant proteins

The GST–rHNF-4$\alpha$1(LBD) recombinant protein was prepared as previously described [3]. The full-length rHNF-4$\alpha$1 was derived by incubating His-rHNF-4$\alpha$1 [7] (10 $\mu$g) with 0.19 units of human thrombin in 20 $\mu$L of 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 2.5 mM $CaCl_2$ for 30 min at 25° followed by the addition of 80 ng of antithrombin III and a further incubation for 5 min at room temperature.

### 2.2. PP-CoA thioesters in cultured cells

Cultured COS-7 cells ($20–30 \times 10^6$) were washed with saline and scraped into methanol. The dry residue was extracted with chloroform:methanol:$H_2O$ 4:2:1.5 (Folch extraction). The interphase protein was further extracted with methanol:2 M ammonium acetate 4:1, and the extract combined with the upper Folch phase was evaporated to dryness, suspended in 50 mM Tris–HCl buffer pH 8.0, and loaded onto a solid-phase C18 cartridge (Varian) equilibrated with 50 mM Tris–HCl pH 8.0. The column was washed with water and the acyl-CoAs were eluted in methanol and subjected to m/e 339 parent ion scanning within the 450–720 m/e range using negative ESI/MS/MS.

### 2.3. Liver acyl-CoA thioesters

Freeze-clamped livers were extracted essentially as described in [8] and the extract loaded onto an oligonucleotide purification cartridge. Acyl-CoAs were eluted by 0.4 mL of 60% acetonitrile in 100 mM $KH_2PO_4$, dried under nitrogen, and suspended in 200 $\mu$L of 2-propanol: 1 mM acetic acid 8:2. The salt precipitate was discarded and the $N_2$-dried extract was resuspended in 150 $\mu$L acetonitrile:$H_2O$ 6:4 and subjected to m/e 339 parent ion scanning within the 450–580 m/e range using negative ESI/MS/MS.

### 2.4. ESI/MS/MS

Samples were analyzed in the negative mode by the VG Quatro Tandem ESI/MS/MS mass spectrometer using cone voltage of 35 V and collision energy of 30 eV. The electrospray source temperature was maintained at 75°, and argon gas pressure inside the collision cell was adjusted to $5.5 \times 10^{-3}$ mbr. The mass spectrometer was operated first in the parent ion scan followed by quantitation of individual acyl-CoAs by the Multiple Reaction Monitoring (MRM) mode, with 15 channels selected and set to predefined nominal m/e values. MRM values for xenobiotic acyl-CoAs were normalized by spiking with authentic acyl-CoAs. Data were processed by the Masslynx program.

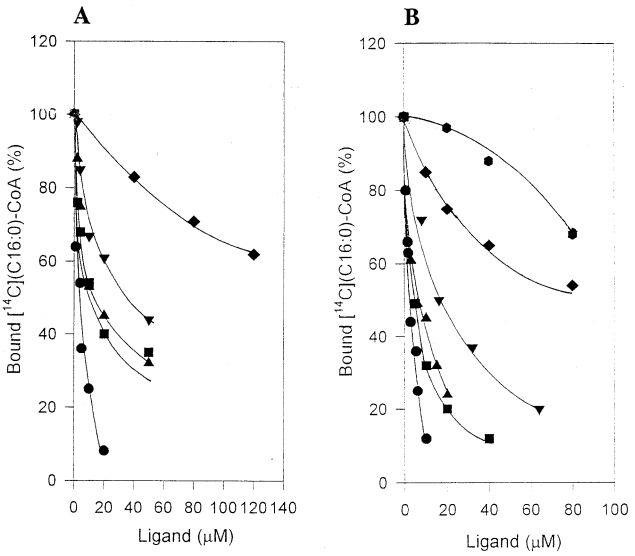


Fig. 1. Acyl-CoA thioesters of hypolipidemic peroxisome proliferators are ligands of HNF-4$\alpha$. Binding of (C16:0)-CoA (●), M14-CoA (▼), M16-CoA (■), M18-CoA (▲), nafenopin-CoA (◆) or bezafibroyl-CoA (⬣) to full-length HNF-4$\alpha$ (A) or GST–HNF-4$\alpha$(LBD) (B) was determined by incubating 100 pmol of the respective recombinant protein with 24 pmol [$^{14}$C](C16:0)-CoA (57 mCi mmol$^{-1}$) and with increasing non-labeled acyl-CoA [3]. One hundred percent specific binding represents 8 pmol of [$^{14}$C](C16:0)-CoA. One representative experiment out of 4–5 independent experiments for each acyl-CoA. $EC_{50}$ values (effective concentrations resulting in 50% specific competition) of M14-CoA, M16-CoA, and M18-CoA for the full-length HNF-4$\alpha$ amounted to 30 ± 5, 11 ± 3, and 14 ± 4 $\mu$M respectively, as compared with 4.0 ± 1.5 $\mu$M for palmitoyl-CoA. $EC_{50}$ values of M14-CoA, M16-CoA, M18-CoA, nafenopin-CoA, and bezafibroyl-CoA for GST–HNF-4$\alpha$(LBD) amounted to 16.0 ± 3.0, 4.5 ± 1.0, 5.3 ± 1.4, 71 ± 10, and >100 $\mu$M, respectively, as compared with 1.8 ± 0.2 $\mu$M for palmitoyl-CoA.

### 2.5. Statistics

Significance was evaluated by applying multiple comparison one-way Anova and then by analyzing the difference between means using Student–Newman–Keuls test.

## 3. Results and discussion

### 3.1. Binding studies

Binding of acyl-CoA thioesters of hypolipidemic PP to full-length recombinant HNF-4$\alpha$ or to the LBD of HNF-4$\alpha$ fused to GST (GST–HNF-4$\alpha$[LBD]) was evaluated by competition with [$^{14}$C]-palmitoyl-CoA binding (Fig. 1, A and B). The apparent binding affinity of the CoA thioesters of MEDICA homologues was in the range of 5–15 $\mu$M i.e. one order of magnitude higher as compared with fibrate-CoA thioesters. Binding of xenobiotic acyl-CoAs was further verified by protection of HNF-4$\alpha$ from limited proteolysis by chymotrypsin [9] (Fig. 2). Binding and protection were specific for the acyl-CoAs, whereas the respective xenobiotic free acids or free CoA did not bind or protect. Further-

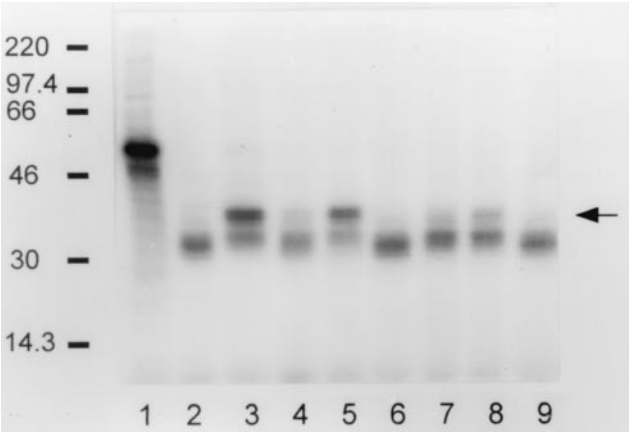


Fig. 2. HNF-4$\alpha$ protection from limited proteolysis by its acyl-CoA ligands. [$^{35}$S]Methionine-labeled rHNF-4$\alpha$ was synthesized using the rabbit reticulocyte lysate transcription/translation kit TnT/T7(Promega). Lysate (0.4 $\mu$l) was preincubated for 30 min at 22° in 10 mM KPi buffer (pH 7.4) containing 5% glycerol in the absence (lanes 1,2) or presence of 100 $\mu$M myristoyl-CoA (lane 3), 100 and 200 $\mu$M M16-CoA (lanes 4,5), 200 $\mu$M M16 free acid (lane 6), 50 and 100 $\mu$M bezafibroyl-CoA (lanes 7,8), and 100 $\mu$M bezafibrate free acid (lane 9), followed by 10 min chymotrypsin (10 $\mu$g/mL) digestion (lanes 2–9) and analysis by 10% SDS–PAGE. Lane 1: undigested protein. (→): Ligand-protected HNF-4$\alpha$ fragment(s).

more, protection was not observed with either Nonidet P-40 or Triton X-100, thus indicating lack of a detergent effect.

### 3.2. Transcriptional modulation

The effect of HNF-4$\alpha$ xenobiotic ligands on its transcriptional activity was evaluated in COS-7 cells cotransfected with an expression vector for HNF-4$\alpha$ and with a CAT reporter plasmid fused to the HNF-4$\alpha$ cognate enhancer of the human apo C-III gene promoter [3]. Incubating the cells in the presence of added M16, M18, nafenopin or bezafibrate free acids resulted in dose-dependent suppression of HNF-4$\alpha$ transcriptional activity (Fig. 3A). The specific requirement for intracellular acyl-CoAs to modulate the transcriptional activity of HNF-4$\alpha$ in transfection studies was verified by the lack of effect of Cl-DICA (HOOC—$CCl_2$—$(CH_2)_{10}$—$CCl_2$—COOH) (Fig. 3A), which due to its interfering $\alpha$-chlorine atoms does not serve as substrate for CoA thioesterification as shown below. The specific requirement for the CoA thioester was further verified by studying the inhibitory effect of M16 in cells cotransfected with long-chain acyl-CoA synthase (Fig. 3B) or incubated in the presence of added triacsin C (Fig. 3C), serving as inhibitor of long-chain acyl-CoA synthase [10]. Suppression of HNF-4$\alpha$ transcriptional activity by M16 was more pronounced and required lower concentrations of the added M16 free acid in cells cotransfected with long chain fatty acyl-CoA synthase, while being partially eliminated by added triacsin C, thus indicating that the inhibitory effect of xenobiotic amphipathic carboxylates in transfected cells was limited by their intracellular conversion to the respective acyl-CoA.

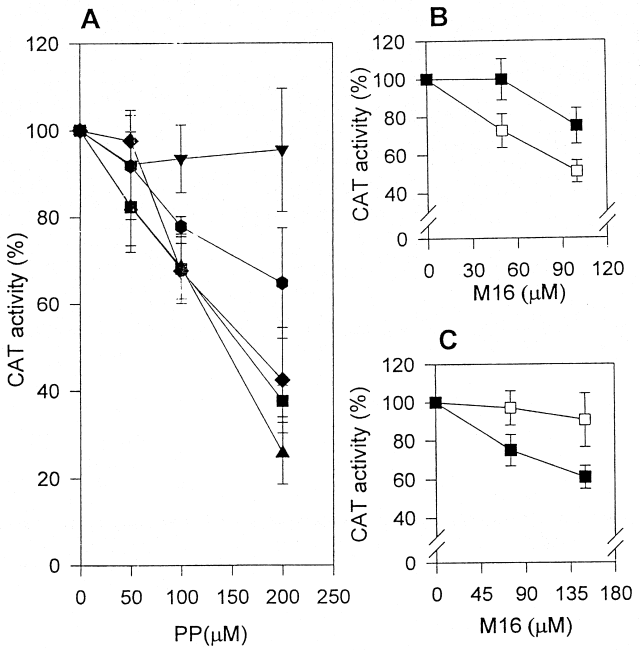


Fig. 3. Suppression of HNF-4$\alpha$ activity by hypolipidemic PP in transient transfection. (**A**) COS-7 cells cotransfected for 6 hr with $(C3P)_3$-tk(thymidine kinase)-CAT [3] (5 $\mu$g) and with either pSG5–HNF-4$\alpha$ [12] (0.2 $\mu$g) or pSG5 (0.2 $\mu$g) were cultured for 40 hr in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and increasing concentrations of M18 (▲), M16 (■), nafenopin (◆), bezafibrate (●), or Cl-DICA (▼) free acids. Percentage CAT activity refers to HNF-4$\alpha$-dependent CAT activity, where 100% activity amounts to 22.4 ± 2.8-fold activation in cells transfected with pSG5–HNF-4$\alpha$ as compared with pSG5. Mean ± SD for 3–4 independent experiments for each PP. (B) COS-7 cells transfected as described in (A) were cotransfected with an expression vector encoding long-chain fatty acyl-CoA synthase [3] (2 $\mu$g) (□) or the empty vector (■). One hundred percent CAT activity amounts to 9.9 ± 2.9-fold activation in cells transfected with pSG5–HNF-4$\alpha$ as compared with pSG5. Mean ± SD for 3 independent experiments. (C) HepG2 cells cotransfected with human (−854/+22) apo C-III–CAT [12] (5 $\mu$g) and with either pSG5–HNF-4$\alpha$ [12] (0.2 $\mu$g) or pSG5 (0.2 $\mu$g) were cultured with increasing concentrations of M16 in the absence (■) and presence (□) of triacsin C (10 $\mu$M). One hundred percent CAT activity amounts to 3.6 ± 0.3-fold activation in cells transfected with pSG5–HNF-4$\alpha$ as compared with pSG5. Mean ± SD for 3 independent experiments.

### 3.3. Intracellular xenobiotic acyl-CoAs

Intracellular acyl-CoAs were identified by negative ESI/MS/MS mass spectrometry as parent ions of the dehydrated phosphopantetheine fragment (m/e 339) common to all acyl-CoAs [11]. The three MEDICA homologues or nafenopin added to COS-7 cells cultured under transfection conditions as described above yielded parent ions of 653.6, 681.7, 709.6, and 649.7 m/e, representing the hydrated acylphosphopantetheine fragments generated from the molecular ions of M14-CoA, M16-CoA, M18-CoA, and nafenopin-CoA, respectively, by loss of 3′,5′-diphosphoadenosine (m/e 426) (Fig. 4). The liver acyl-CoA profile in rats treated with hypolipidemic PP was determined by negative ESI/MS/MS mass spectrometry under conditions where acyl-CoA molecular ions could be identified (Fig.

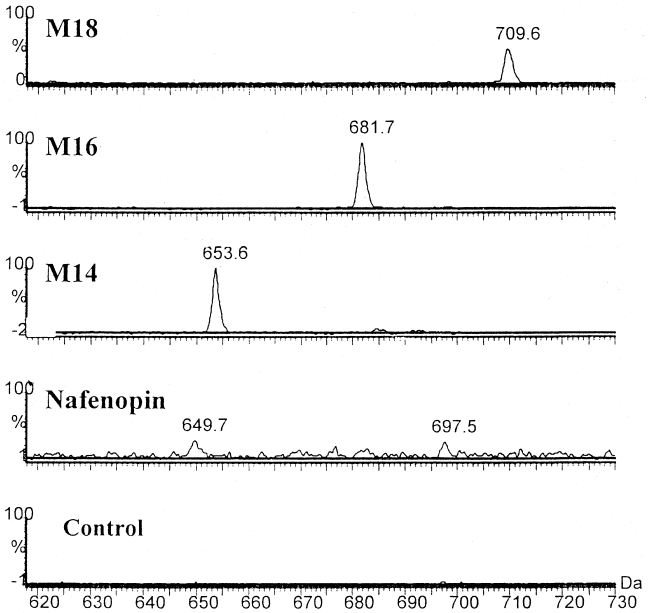


Fig. 4. PP-CoA thioesters in cultured cells. COS-7 cells (20–30 × $10^6$) were cultured with 200 $\mu$M M18, M16, M14, or nafenopin as described in Fig. 3. PP-CoA thioesters in cultured cells were determined as described in Methods. One representative experiment out of 5 independent experiments for each PP.

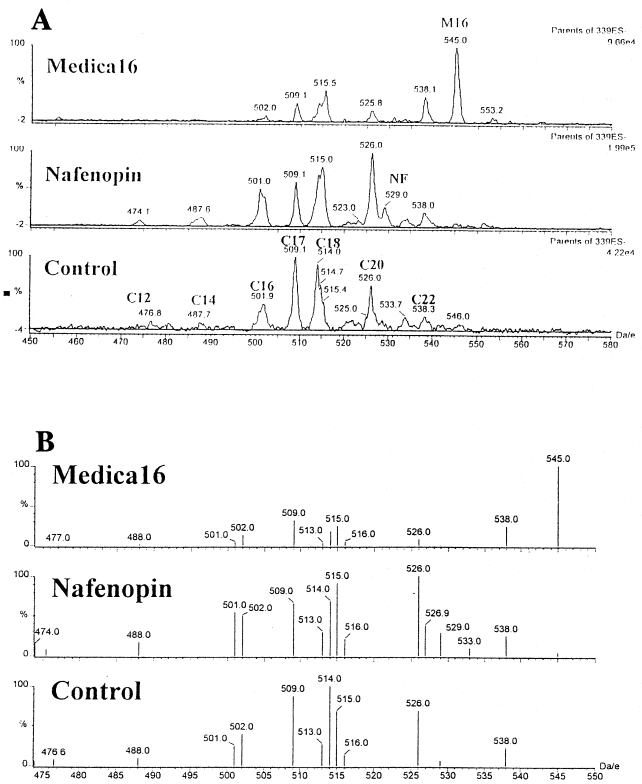


Fig. 5. Liver endogenous fatty acyl-CoA and xenobiotic PP-CoA thioesters in rats treated with hypolipidemic PP. (A) Male albino rats weighing 150–200 g were treated for 3 days with 0.25% (w/w) M16, 0.25% (w/w) nafenopin, or 0.5% (w/w) bezafibrate mixed in the diet. Acyl-CoA thioesters were determined in freeze-clamped livers as described in Materials and Methods. (C17:0)-CoA (20 nmol) (m/e 509.1) served as internal standard. (B) Endogenous fatty acyl-CoA and xenobiotic acyl-CoA molecular ions determined as described in (A) were quantified by their respective Multiple Reaction Monitoring (MRM) values. MRM values for xenobiotic acyl-CoAs were normalized by the (C17:0)-CoA internal standard and further normalized by spiking with authentic acyl-CoAs. Total fatty acyl-CoAs in non-treated animals amounted to 68.0 ± 4.8 nmol/g liver. Xenobiotic acyl-CoAs amounted to 112.7 ± 23.9, 100.1 ± 30.0, and 40.0 ± 4.5 nmol/g (mean ± SEM [N = 3–5]) for M16-CoA, nafenopin-CoA, and bezafibroyl-CoA (not shown), respectively.

5A) and quantified (Fig. 5B). Treatment with M16 resulted in a dramatic increase in M16-CoA (m/e 545) content that amounted to about 2-fold total fatty acyl-CoA content of non-treated animals, thus indicating its dominant abundance as compared with any other endogenous fatty acyl-CoA. The increase in liver M16-CoA was accompanied by the appearance of a nuclear M16-CoA (not shown). Similarly, treatment with nafenopin or bezafibrate resulted in a significant increase in liver nafenopin-CoA (Fig. 5, A and B) or bezafibroyl-CoA (not shown), respectively. The CoA thioesters of Cl-DICA or its related daughter ions were absent in the ESI/MS/MS spectra of cells incubated with Cl-DICA up to 200 $\mu$M or in livers of rats treated with Cl-DICA up to 0.4% (w/w) for 14 days, in line with the lack of effect of Cl-DICA in HNF-4$\alpha$ transfection studies (Fig. 3A).

### *3.4. HNF-4α/PPARα interplay in rodents and human liver cells*

Inhibition of HNF-4$\alpha$ activity by PP capable of being thioesterified *in vivo* into their respective CoA thioesters as reported here offers a direct mode of transcriptional suppression of HNF-4$\alpha$-controlled genes in addition to the previously reported indirect mode due to HNF-4$\alpha$ displacement from its cognate enhancer by non-productive binding of PP-activated PPAR$\alpha$/retinoid X receptor [12] (Fig. 6). Since the direct and indirect modes require the CoA thioester (Fig. 3) and the free acid [13,14] of a given PP, respectively, and as Cl-DICA does not serve as substrate for CoA thioesterification, suppression of HNF-4$\alpha$-responsive genes (e.g. apo C-III) by Cl-DICA is PPAR$\alpha$-specific, whereas suppression by MEDICA homologues or fibrates may be mediated both by the indirect PPAR$\alpha$ and the direct HNF-4$\alpha$ pathways. The contribution made by each may depend on the prevailing composition of nuclear PP-CoAs as compared with the respective free PP as well as on the prevailing content and transcriptional activity of PPAR$\alpha$ and HNF-4$\alpha$ within a specific cell type.

The role played by the indirect PPAR$\alpha$ pathway in suppressing the transcription of apo C-III by hypolipidemic PP in rodents [12,15,16] was evaluated by exploiting the PPAR$\alpha$ specificity of Cl-DICA. Thus, treating rats with Cl-DICA or M16 results in a decrease in liver apo C-III mRNA, plasma apo C-III, plasma VLDL half-life, and plasma lipids (Table 1), with a concomitant induction of peroxisomal activities. Furthermore, the $\beta,\beta'$-methyl-substituted and $\alpha,\alpha'$-chloro-substituted dioic acids similarly

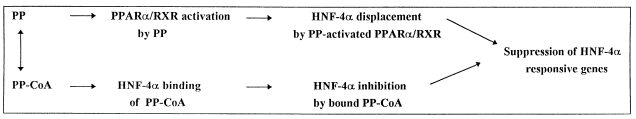


Fig. 6. Suppression of HNF-4$\alpha$-responsive genes by peroxisome proliferators. Suppression of HNF-4$\alpha$-responsive genes by PP may be mediated directly or indirectly by HNF-4$\alpha$ suppression (lower line) or by PPAR$\alpha$ activation (upper line), respectively. The indirect pathway requires the free PP acid and is mediated by HNF-4$\alpha$ displacement of its cognate enhancer by binding of PP-activated PPAR$\alpha$/RXR (retinoid X receptor) under conditions where PPAR$\alpha$/RXR binding is transcriptionally non-productive. The direct pathway requires PP-CoA and is mediated by PP-CoA inhibition of the transcriptional activity of HNF-4$\alpha$. The two pathways may either complement or prevail independently of each other in respective species.

activate PPAR$\alpha$ in COS-7 cells cotransfected with a PPAR$\alpha$ expression plasmid and a CAT reporter plasmid promoted by the PPAR$\alpha$ cognate enhancer of the peroxisomal acyl-CoA oxidase (AOX) promoter [13] (not shown). Since Cl-DICA fails to yield the respective acyl-CoA and therefore to suppress HNF-4$\alpha$ (Fig. 3A), suppression of liver apo C-III transcription by Cl-DICA in rats is HNF-4$\alpha$-independent and may be accounted for by PPAR$\alpha$ activation. Hence, the hypolipidemic activity of peroxisome proliferators (e.g. fibrates, MEDICA homologues, Cl-DICA) is mediated in rodents by the PPAR$\alpha$ transduction pathway.

Evaluation of the PPAR$\alpha$-independent pathway in rodents was attempted in PPAR$\alpha$ knockout [17] as compared with PPAR$\alpha$ +/+ mice treated with M16 or bezafibrate. However, doses required to decrease plasma triglycerides by M16 or bezafibrate in PPAR$\alpha$ +/+ mice were exceptionally high ($\geq$0.5% [w/w]), and the observed lipid-lowering effect of both was unaccompanied by a decrease in liver apo C-III mRNA. Moreover, liver steady-state M16-CoA levels in PPAR$\alpha$ knockout mice were 5-fold lower as compared with liver M16-CoA levels in rats treated with similar doses, thus limiting the availability of the putative acyl-CoA ligands of HNF-4$\alpha$ in PPAR$\alpha$ knockout mice treated with hypolipidemic PP. The decrease in liver acyl-CoAs presumably reflects the lower expression of the long chain fatty acyl-CoA synthase gene in PPAR$\alpha$ knockout mice [18]. Hence, the species background and the suppressed acyl-CoA synthase in PPAR$\alpha$ knockout mice made it impossible to evaluate the contribution made by the PPAR$\alpha$-independent pathway to the hypolipidemic effect of PP in rodents.

Table 1
Hypolipidemic effect of Cl-DICA in rats

| | Nontreated | Cl-DICA | M16 |
|---|---|---|---|
| Plasma triglycerides (mg %) | 61.0 ± 13.5 | 16.8 ± 1.9* | 12.7 ± 4.0* |
| Plasma VLDL triglyceride clearance ($min^{-1}$) | 0.07 ± 0.01 | 0.17 ± 0.02* | 0.43 ± 0.11* |
| Plasma apo C-III (mg %) | 33 ± 10 | 7 ± 2* | 11 ± 4* |
| Liver apo C-III mRNA[a] | 1.0 ± 0.2 | 0.4 ± 0.1* | 0.3 ± 0.3* |

Male albino rats were treated for 5 days with 0.09% (w/w) of either M16 or Cl-DICA mixed in the diet non-fasting plasma triglycerides, plasma VLDL triglyceride clearance, plasma apo C-III, and liver apo C-III mRNA were determined as previously described [12,16]. Mean ± SEM (N = 3–5).

[a] Non-treated liver mRNAs were arbitrarily presented as 1.0.

* Significantly differs from the respective non-treated value by one-way ANOVA ($P < 0.05$).

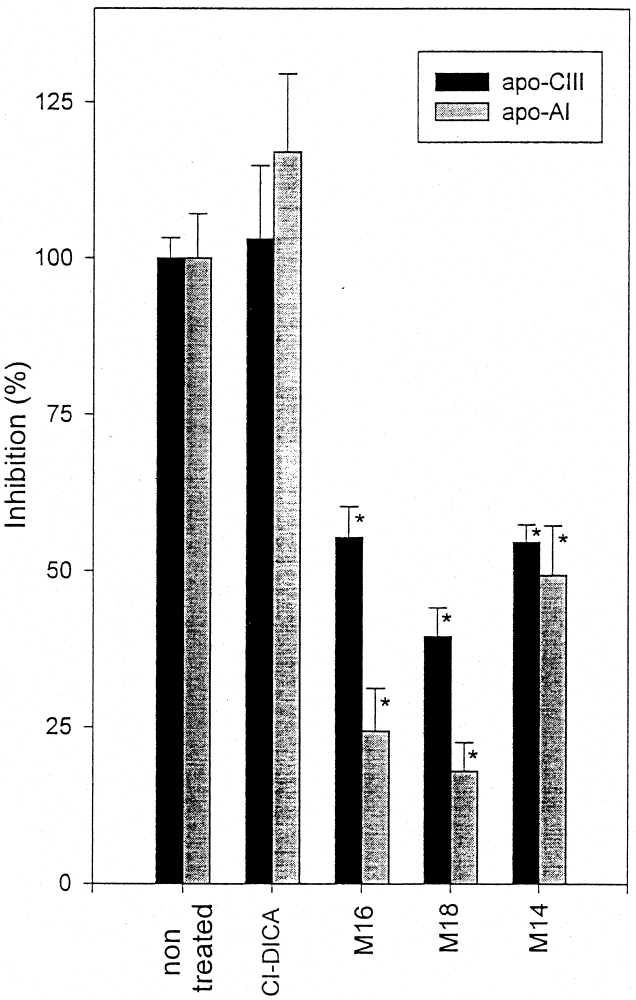


Fig. 7. Suppression of apo C-III and apo A-I mRNAs by HNF-4$\alpha$ xenobiotic ligands in human liver cells. Apo C-III and apo A-I mRNA were determined in HepG2 cells incubated for 48 hr in the absence or presence of 250 $\mu$M each of Cl-DICA, M16, M18, or M14 as previously described [12]. Hep G2 apo C-III mRNA was probed using the 500-bp *pst*I restriction fragment of h-apo C-III cDNA. HepG2 apo A-I mRNA was probed using the 900 base pair *pst*I restriction fragment of h-apo A-I cDNA. The extent of hybridization was normalized to the signal obtained with glyceraldehyde 3-phosphate dehydrogenase (GAP). Mean ± SEM of the apo C-III mRNA/GAP mRNA ratio and of the apo A-I mRNA/GAP mRNA ratio for 4 independent experiments. *Significantly differs from the respective non-treated value by one-way ANOVA ($P < 0.05$).

The roles played by the indirect PPAR$\alpha$ and the direct HNF-4$\alpha$ pathways in humans were evaluated by studying the effect of MEDICA homologues as compared with Cl-DICA on mRNA levels of HNF-4$\alpha$-responsive genes (e.g. apo C-III, apo A-I) in human liver cells. In contrast to MEDICA homologues, which yield the respective CoA thioesters and suppress the expression of the HNF-4$\alpha$-re-

sponsive apo C-III and apo A-I genes in HepG2 cells, Cl-DICA was ineffective (Fig. 7). Hence, the hypolipidemic effect of PP in humans based on transcriptional suppression of liver apo C-III [12,15] is PPAR$\alpha$-independent and mediated by the PP-CoA/HNF-4$\alpha$ transduction pathway. Plasma apo C-III suppression by MEDICA homologues as contrasted with the hypolipidemic inefficacy of Cl-DICA was confirmed in human clinical trials.[1]

None of the characteristic liver activities induced by PPAR$\alpha$ activators (PP) in rodents (e.g. peroxisome proliferation, peroxisomal $\beta$-oxidation activities, cytochrome P-450) are induced in humans chronically treated with hypolipidemic PP [19,20]. The molecular basis for the lack of effect of PPAR$\alpha$ activators in the human liver still remains to be investigated. As long as the hypolipidemic activity of PP was exclusively ascribed to PPAR$\alpha$ activation [12,15], the discrepancy between the hypolipidemic efficacy of PP in humans as opposed with their inefficacy as PPAR$\alpha$ activators in the human liver remained unsolved. The direct PP-CoA/HNF-4$\alpha$ pathway offers a hypolipidemic mode of action for PP in humans independent of PPAR$\alpha$, thus explicitly dissociating between the HNF-4$\alpha$-mediated hypolipidemic and the PPAR$\alpha$-mediated "peroxisome proliferative" activities of hypolipidemic PP.

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