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Regulation of CYP4A1 and Peroxisome Proliferator-activated Receptor Alpha Expression by Interleukin-1β, Interleukin-6, and Dexamethasone in Cultured Fetal Rat Hepatocytes

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ABSTRACT. The CYP4A1 isoenzyme induced in rodents by peroxisome proliferators is known to be repressed at a pretranslational level by interferon. Interleukin-1β (IL-1β) also reduces CYP4A1-related 12-laurate hydroxylase activity in cultured fetal rat hepatocytes after induction by clofibric acid. In this fetal hepatocyte model, IL-1β and interleukin-6 (IL-6) were tested for their ability to reduce 12-laurate hydroxylase activity, CYP4A1 apoprotein content, and the CYP4A1 mRNA level. IL-1β and IL-6 strongly diminished CYP4A1 activity and apoprotein and mRNA levels in a dose- and time-dependent manner. CYP4A1 expression is thus down-regulated at a pretranslational level by these cytokines. As it has been shown that the peroxisome proliferator-activated receptor alpha (PPARα) mediates the induction of the CYP4A1 gene by a peroxisome proliferator, the capacity of IL-1β or IL-6 to modulate the PPARα mRNA level was tested. It was found that IL-1β and IL-6 both repress the induction of PPARα expression exerted by the combined action of clofibric acid and dexamethasone. However, even at the highest concentration (10 ng/mL) tested for both cytokines, IL-1β as well as IL-6 failed to abolish the induction of CYP4A1 by dexamethasone. The mechanism of the protective effect of the synthetic glucocorticoid on CYP4A1 repression by interleukins is discussed. BIOCHEM PHARMACOL 54;8:889–898, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. CYP4A1; PPARα; interleukins; dexamethasone; fetal rat hepatocytes

Inflammation, infection, and tissue injuries alter the organism's physiological homeostasis and induce the acute phase response in the liver. Interleukin-1β (IL-1β),[‖] interleukin-6 (IL-6), tumor necrosis factor-alpha (TNFα), and interferons are the major mediators involved in the acute phase response [1, 2]. These cytokines exert closely related effects on the liver, including the release of acute phase proteins from hepatocytes, decreased albumin production, and diminished expression of drug-metabolizing enzymes. IL-6 is the major component of the acute phase response, and the effect of IL-1 on hepatocytes is partially mediated by IL-6 [3].

Cytochromes P450 (CYP) (EC 1.14.14.1) are drug-metabolizing enzymes that catalyze the monooxygenation of a broad spectrum of xenobiotics and endogenous compounds [4]. Expression of CYP isoforms is altered by inflammatory stimuli and agents mimicking infection [5]. Depression of ethoxycoumarin deethylase activity by lipopolysaccharides (LPS) in the liver is mediated by IL-1, as shown in endotoxin-resistant mice [6]. Chen *et al.* [7] suggested that the *in vivo* effects of LPS, TNFα, and IL-1β on CYP-related activities were partially mediated by IL-6. *In vitro*, IL-6 decreases the levels of phenobarbital (PB)-induced CYP2B mRNA and protein in cultured rat hepatocytes [8, 9]. Furthermore, in primary cultures of adult rat hepatocytes, phenobarbital induction of CYP1A, 2B, 2C, 2E, and 3A mRNAs is antagonized by IL-1β [10]. In HepG2 hepatoma cells, IL-6 suppresses 3-methylcholanthrene induction of CYP1A1 [11], as does IL-1β in isolated hepatocytes [12]. IL-1β, IL-6, and TNFα depress CYP1A, CYP2E1, and CYP3A mRNAs in cultured adult human hepatocytes [13]. The inhibition of several CYP-dependent activities by IL-1β has also been assessed in cultured fetal rat hepatocytes [14].

Rat CYP4A isoforms catalyze the hydroxylation of fatty

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‖ *Abbreviations:* CA, clofibric acid; CYP, cytochrome P450; D, dexamethasone; IL-1β, interleukin-1β; IL-6, interleukin-6; LAH, lauric acid 12-hydroxylase; LPS, lipopolysaccharide; NF-IL6, nuclear factor-IL6; PB, phenobarbital; PPAR, peroxisome proliferator-activated receptor; RT-PCR, reverse transcription-polymerase chain reaction; TNFα, tumor necrosis factor α.

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acids and eicosanoids at the terminal position and are induced by peroxisome proliferators [15, 16]. Clofibric acid (CA), a peroxisome proliferator, induces CYP4A expression both *in vivo* and *in vitro* [17]. The induction of the CYP4A1 gene is mediated by a specific receptor, the peroxisome proliferator-activated receptor (PPAR) [18]. Thus far, three subtypes of PPARs have been described in vertebrates: PPARα, β (also called δ or NUC-1), and γ [19]. They exhibit specific tissue distributions [20]. These nuclear receptors were first reported to be activated by substances that induce peroxisomal proliferation. Further investigations revealed that natural fatty acids and prostaglandins are also potent activators of PPARs. The effects of several hormones, including glucocorticoids, on PPARα expression have been reported [21, 22], but those of cytokines have yet to be elucidated. The interferon-α/β inducer, polyinosinic acid-polycytidylic acid, depresses the CYP4A mRNA level in clofibrate-treated rats [23]. In previous studies, we have shown that IL-1β inhibits clofibric acid-induced expression of the major CYPs including CYP4A1 in cultured fetal rat hepatocytes [24, 25]. In this system, dexamethasone (D), a synthetic glucocorticoid, is required to promote and maintain induction of CYP4A by clofibric acid. In fact, glucocorticoids are used at low doses in practically all cell culture media. This required presence of dexamethasone raises the question of the involvement of glucocorticoids in the regulation of CYP (especially CYP4A1) expression in the liver. CYP2C11 and CYP2C12 are down-regulated by low doses of dexamethasone in male and female rats, respectively, and IL-1 strengthens this repression [26]. On the other hand, high doses of dexamethasone induce CYP1A-, CYP2B-, and CYP3A-related activities, and several isoforms are protected from the IL-1β effect by this glucocorticoid [27]. Thus, the role of dexamethasone in CYP repression by cytokines requires further examination.

In the present study, we have examined the repressive effects of IL-6 and IL-1β on CYP4A1 induction by clofibric acid. The results presented here demonstrate the IL-1β and IL-6 decreased the lauric acid 12-hydroxylase (LAH) activity, CYP4A1 apoprotein content, and the CYP4A1 mRNA level. The time course of action of both interleukins on LAH activity has also been studied. PPARα was involved in the mechanisms of CYP4A1 induction by dexamethasone and/or clofibric acid and in the mechanism of CYP4A1 repression by IL-1β or IL-6. Finally, a protective effect of dexamethasone against CYP4A1 repression by IL-1β, probably associated with an increase in PPARα synthesis, was established, confirming previous findings on other CYP isoforms in the same model [14, 24].

MATERIALS AND METHODS

Reagents and Chemicals

The culture medium used was minimum essential medium (Techgen, Zellich, Belgium) supplemented with 10% newborn calf serum, 100 units/mL penicillin/streptomycin, and 250 μg/mL fungizone (Gibco BRL, Grand Island, NY, USA). Dexamethasone, clofibric acid, lauric acid, and 4-chloro-napthtol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Recombinant human interleukin-6 and DNA molecular weight marker III were from Boehringer Mannheim (Mannheim, Germany); phenobarbital was from Merck (Hohenbrunn, Munich, Germany), molecular mass markers (prestained SDS-PAGE standards, low range) were from Bio-Rad (Richmond, CA, USA), and [^{14}C]lauric acid was from Amersham (Arlington Heights, IL, USA). Recombinant human interleukin-1β was a generous gift from Dr. B. Terlain (Rhone-Poulenc-Rorer, Vitry/Seine, France). Other reagents were of analytical grade.

Animals

Male and pregnant female Sprague Dawley rats were obtained from IFFA-CREDO (Lyon, France) and used as a control for Western and Northern blot experiments. Clofibrate was suspended in corn oil (200 mg/mL). Phenobarbital (50 mg/kg in PBS; twice a day for 4 days) and clofibrate (400 mg/kg, 3 days) were injected intraperitoneally.

Cell Culture

Hepatocytes from 18-day-old rat fetuses were prepared according to Bolline *et al.* [28] and cultured as previously described [24]. Cells were cultured for 96 hr, and the culture medium was changed every 24 hr. All treatments were started 24 hr after seeding unless otherwise stated. Dexamethasone (10^{-6} M), clofibric acid (10^{-3} M), and phenobarbital (2×10^{-3} M) were applied alone or in combination. Dexamethasone and clofibric acid were dissolved in DMSO, leading to final DMSO concentrations in the culture media of 0.002 and 0.2%, respectively, concentrations which do not induce CYP expression. Phenobarbital and interleukins were dissolved in serum-free culture medium. In time-course experiments, IL-1β or IL-6 was added after pretreatment of the hepatocytes with clofibric acid and dexamethasone and 24 hr before the cells were scraped. Assays concerning LAH activity, CYP4A1 protein, and mRNA as well as PPARα mRNA were carried out with the same cultured cells.

Determination of Enzyme Activities

LAH activity was determined according to the method of Parker and Orton [29], 11- and 12-hydroxylauric acid being separated by thin layer chromatography. Metabolities were quantified with a ray test apparatus (LB276, Berthold, Belgium) and Rita software (Berthold) [24]. Protein concentration was determined according to Lowry *et al.* [30].

Western Immunoblot Analysis

CYP4A1 immunoblotting was carried out on cell microsomal proteins. Cells from ten culture dishes were homogenized with cold PBS. Microsomes were prepared as previously described [31] and treated for immunoblotting according to Kremers *et al.* [32]. Liver microsomes from clofibrate-treated male rats were used as a control. Microsomal proteins (2 μg per lane) were separated using 10% SDS-PAGE according to Laemmli [33]. Western blotting was performed according to Towbin *et al.* [34]. CYP4A1 protein was detected by incubation with a specific goat antirat polyclonal antibody, kindly provided by Prof. G. G. Gibson (University of Surrey, Guildford, UK) and then with rabbit antigoat IgG linked to peroxidase (Prosan, Gentbrugge, Belgium). The reaction was developed with 4-chloronaphthol. The antirat CYP4A1 antibody recognizes a single protein in cultured cells. Band intensities were measured by densitometry and expressed as percentages of a control value measured on protein from dexamethasone plus clofibric acid-treated cells.

Isolation of RNA and RNA Blot Analysis

Total cytoplasmic RNAs were prepared according to Gough [35]. RNAs were purified with DNase I, RNase-free (Boehringer, Mannheim) according to the manufacturer's instructions. Three phenol/chloroform extractions ensured elimination of possible residual contaminant DNA.

cDNAs corresponding to parts of the rat CYP4A1 and PPARα, respectively, were obtained by reverse transcription coupled to polymerase chain reaction (RT-PCR) according to Jones [36]. The first cDNA strand of the rat CYP4A1 was synthesized from 2 μg of total RNAs using the superscript II RNase H-reverse transcriptase (Gibco BRL) and the primer (5′-AGAGAGACTATGCAGGGGAGA-3′) from Eurogentec (Seraing, Belgium), hybridizing to the sequence located between nucleotides 1971 and 1991 [37]. The first cDNA strand was purified with the Glass Max Kit (Gibco BRL). PCR amplification was performed with the primers 5′-CCATGTGTATCAAGGAGGCCC-3′ (nucleotides 1127-1147) and 5′-AGAAGAGCGGGTATGGGAAGG-3′ (nucleotides 1711-1731) of rat CYP4A1 cDNA [37] using the Gene Amp PCR kit (Perkin-Elmer Cetus, Norwalk, CT, USA). The following PCR program was used: 30 cycles at 94° for 1 min, 60° for 1 min, and 72° for 2 min. The PCR-amplified DNA fragment was analyzed by 2 % (w/v) agarose gel electrophoresis in 40 mM Tris-HCl buffer, pH 7.5, containing 20 mM acetic acid and 1 mM EDTA. The 604-bp PCR-amplified DNA fragment was purified with the prep A gene DNA purification matrix kit (Bio-Rad). The cDNA probe for rPPARα was produced by RT-PCR from 10 μg of rat liver total RNAs. The primers used, derived from the mouse PPARα sequence [38], were α-down 5′-TTCACATGCGTGAACTTCGTAGTGGTACCC-3′ (sequence located at the 3′-end of the A/B domain) and α-up 5′-CTGTGGCCTGCCTGGCCACATCCATCCAAC-3′ (sequence -30/-1 located upstream of the A/B domain). The PCR program used was as described above, except that the annealing temperature was 55°. After purification, the PCR-amplified CYPA41 and rPPARα DNA fragments were sequenced [39].

Northern and dot-blot analyses were carried out according to Thomas [40] using nylon membrane (Amersham). Total RNAs were electrophoresed in 1% (w/v) agarose gel in 10 mM phosphate buffer, pH 6.5. Membranes were probed with the corresponding PCR-amplified product labeled with [γ-^{32}P]dCTP according to Feinberg and Vogelstein [41] using the megaprime DNA labeling kit (Amersham). After hybridization, the membranes were washed four times for 5 min in 0.3 M NaCl, 0.03 M sodium citrate at room temperature and once for 15 min at 50° in 0.075 M NaCl, 7.5 mM sodium citrate. Blots were autoradiographed at −80° using Kodak XAR-2 films. Autoradiograms were analyzed with the phosphorimager system, and quantifications were carried out as previously described [42].

Statistical Analysis

LAH activity data were expressed as the mean ± S.D. of three assays. Groups were compared using the Dunnett's test following ANOVA. Data were considered statistically different when $p < 0.05$. The densitometric analyses of CYP4A1 apoprotein content were carried out on six Western blots. Equivalent loading of the PAGE wells was controlled by staining the gels and the blots to minimize intraassay variability. Data from Northern blot experiments were quantitated with a phosphorimager. Messenger RNA levels for CYP4A1 and PPARα were normalized against the level of actin mRNA. Reproducible results were obtained from at least three independent assays.

RESULTS

Repression of LAH Activity by IL-6 or IL-1β

Fetal rat hepatocytes in primary culture were treated by a combination of dexamethasone and clofibric acid to allow maximal induction of LAH activity. The induction factor reached 25-fold at 96 hr. IL-6 was added in the culture medium at concentrations ranging from 0.1 to 20 ng/mL (Fig. 1). LAH induction was diminished by 25% when a 0.1 ng/mL dose of IL-6 was applied. Repression of LAH activity by IL-6 was dose-dependent. It was strongest for a concentration of 20 ng/mL; however, the activity level remained 7.5 times higher than that noted in uninduced control cells.

Repression of LAH activity by either IL-1β or IL-6 was time-dependent. The time course of this repression is illustrated in Fig. 2. LAH activity remained stable for 5 to 6 hr after addition of 10 ng/mL IL-1β or IL-6. Within 7 hr, significant repression appeared, becoming more pronounced by the end of the tenth hour when 80 and 60% repression was observed with IL-1β and IL-6, respectively.

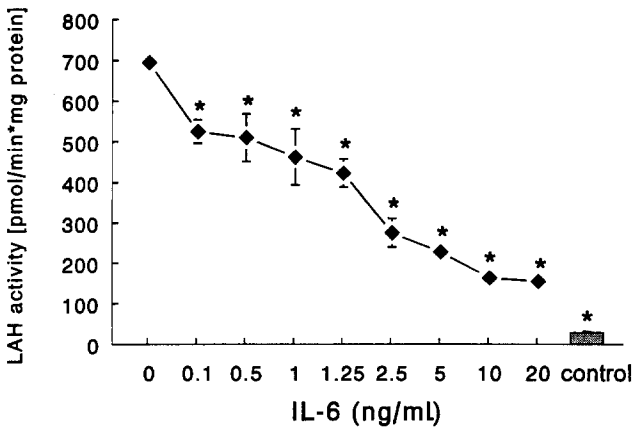

FIG. 1. Effect of IL-6 on LAH activity. The action of increasing IL-6 concentrations on CYP4A-related enzyme activity was studied. Cells were treated with 10^{-6} M dexamethasone (D), 10^{-3} M clofibric acid (CA), and different concentrations of IL-6, all these supplements being added 24 hr after seeding. The medium was renewed every 24 hr. LAH activity (pmol/mn per mg protein) was measured on whole homogenates 72 hr after the beginning of treatment as described under "Materials and Methods." % The control activity was that measured in untreated cells cultured for 96 hr. Each result is expressed as the mean ± S.D. of three assays on homogenates. The absence of visible error has indicates that the S.D. is smaller than the symbol. *, significantly different from D + CA without IL-6 (*, $p < 0.05$).

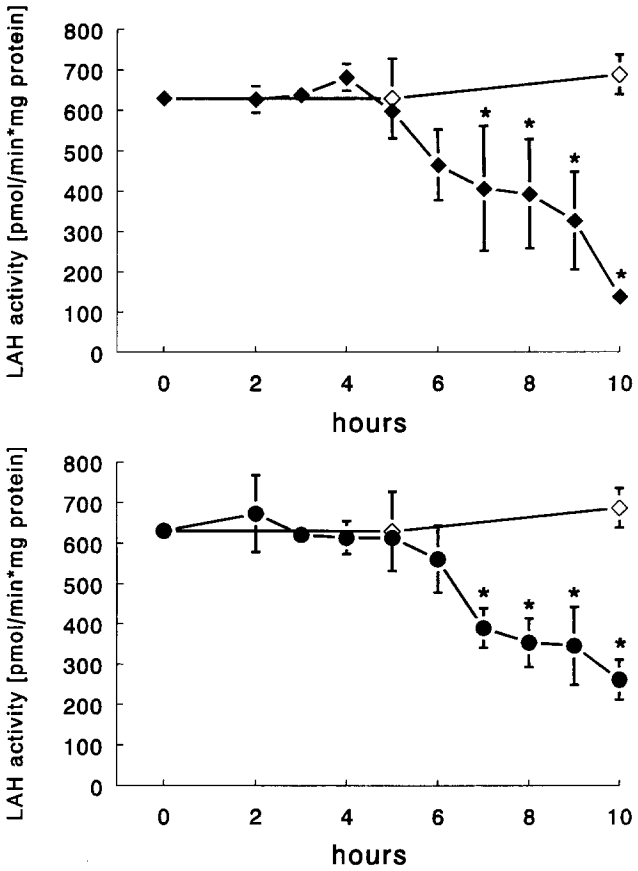

FIG. 2. Time course of the effects of IL-1β and IL-6 on LAH activity. The effects of IL-1β and IL-6 on LAH activity were studied, the cultured cells being left in contact with the cytokine for different periods of time. Cultures were carried out with D (10^{-6} M) + CA (10^{-3} M), and the medium was renewed every 24 hr. IL-1β or IL-6 (10 ng/mL) was added 72 hr after seeding. LAH activity was measured on whole homogenates every hour after the addition of IL-1β or IL-6. Each result is expressed as the mean ± S.D. of three assays on homogenates. The absence of visible error bars indicates that the S.D. is smaller than the symbol. *, significantly different from D + CA at 0 hr (*, $p < 0.05$).

Dexamethasone Reduces Repression

When fetal rat hepatocytes were cultured in the presence of IL-1β alone, LAH activity was under the limit of detection. As previously reported [24], dexamethasone alone did not significantly induce CYP4A1 activity. The combined action of dexamethasone and IL-1β reduced LAH activity under the value obtained when the cells were cultured with the glucocorticoid alone. In addition, treatment with clofibrate and IL-1β resulted in a repression of LAH activity to an extent below that observed in untreated cells (Table 1). This indicates that the fibrate drug did not reverse nor attenuate the repression of LAH activity by IL-1β.

In contrast, dexamethasone altered the pattern of LAH activity induction by clofibric acid and that of repression by IL-1β (Table 1). Cells were treated 24 hrs after seeding and for 72 hr with clofibric acid and/or without IL-1β. Dexamethasone was added 24, 48, or 72 hr after seeding. Since the cells were harvested 96 hr after seeding, the duration of dexamethasone treatment was 72, 48, or 24 hr, respectively. Clofibric acid-induced cells treated for 72 hr with dexamethasone exhibited a significantly higher LAH activity than the corresponding control cells (185%). IL-1β (10 ng/mL) reduced clofibric acid-induced LAH activity in all cases examined.

To determine the degree of repression, we calculated the ratio of unrepressed to repressed clofibric acid-induced activity in each case. This ratio was clearly found to decrease with the duration of treatment, dropping from 46 in the absence of dexamethasone to 13 after a 72-hr treatment with the synthetic glucocorticoid. Thus, dexamethasone reduces repression of LAH activity exerted by IL-1β in a time-dependent manner.

Reduction of CYP4A1 Apoprotein Level by Interleukins

The level of CYP4A1 apoprotein was determined by Western blotting (Fig. 3A). One band was detected with microsomal extracts from cultured cells (lanes 3–17), while two bands were seen with those from clofibrate-treated rat liver (lane 1). This discrepancy is likely due to an isoform immunologically related to CYP4A1, which is not yet expressed during the fetal stage. No band was detected with microsomal proteins extracted from cells cultured in control medium supplemented (lane 4) or not (lane 3) with dexamethasone. Phenobarbital failed to induce CYP4A1 expression in adult (lane 2) and fetal (lane 7) hepatocytes. Our results confirm the induction of CYP4A1 by clofibric acid in fetal rat hepatocytes cultured without or with

TABLE 1. Effect of dexamethasone on LAH activity

Treatment	LAH activity (pmol/mn*mg protein) after dexamethasone treatment lasting (hr)			
	0	24	48	72
CA	418.48 ± 11.58 (1)	416.72 ± 13.5	486.21 ± 19.66	782.80 ± 85.06*
CA+IL-1β	9.20 ± 2.28 (2)	13.51 ± 2.23	19.89 ± 2.25†	62.71 ± 3.60†
Repression ratio	46	31	24	13

The effect of dexamethasone was studied on cells treated with CA (10^{-3} M) alone or with CA + IL-1β (10 ng/mL). Treatment began 24 hr after seeding. Dexamethasone (10^{-3} M) was added 24, 48, or 72 hr after seeding. The medium was renewed every 24 hr. LAH activity was measured on whole homogenates 96 hr after seeding. Results are expressed as the mean ± S.D. of three assays on homogenates. Value for untreated control cells at 96 hr: 28.70 ± 2.92. The "repression ratio" shown below the activity values is the ratio of unrepressed to repressed activity.

* significantly different from v(1), $p < 0.05$.

† Significantly different from (2), $p < 0.05$.

dexamethasone for 96 hr (lanes 5 and 6). Both IL-1β and IL-6 clearly reduced the intensity of the CYP4A1 band in a dose-dependent manner (lanes 10–13 and 14–17). No band was detected when the IL-1β or IL-6 concentration was 10 ng/mL (lanes 13 and 17). The densitometric analyses carried out on the immunoblots confirmed the visual observations (Fig. 3B).

Effects of Interleukins and Dexamethasone on CYP4A1 mRNA

Electrophoresis of PCR-amplified CYP4A1 cDNA revealed the presence of the expected fragment of 604 bp estimated by comparison to the DNA molecular weight markers. The analysis of the 604-bp DNA sequence confirmed the validity of the probe. Northern blot analysis (Fig. 4) revealed a CYP4A1 mRNA transcript with a relative size of 2.0 kb, as expected from earlier work [37]. The abundance of the specific transcript varied according to the culture conditions (Table 2). When either dexamethasone (Fig. 4, lane 2) or clofibric acid (lane 3) was added alone, each was found to increase the level of CYP4A1 mRNA in the cultured cells. However, induction was maximal only when both inducers were added together (lane 4). Under these conditions, IL-1β (lanes 5 and 6) as well as IL-6 (lanes 7 and 8) reduced the level of CYP4A1 mRNA. The repressive action of IL-6 was more efficient than that of IL-1β. Surprisingly, in the presence of dexamethasone alone, IL-1β (lane 9) and IL-6 (lane 10) failed to reduce the mRNA even at the highest concentration tested (10 ng/mL). The CYP4A1 mRNA level was higher in adult rat liver cells (lane 11) than in induced cultured fetal rat hepatocytes (lane 4).

Effects of Inducers and Interleukins on PPARα mRNA Level

The amount of PPARα mRNA was evaluated by dot-blotting (Fig. 5 and Table 3) and Nothern (Fig. 6 and Table 4) blotting. As a first step in the expression analyses of PPARα mRNA, the specificity of the rat PPARα DNA

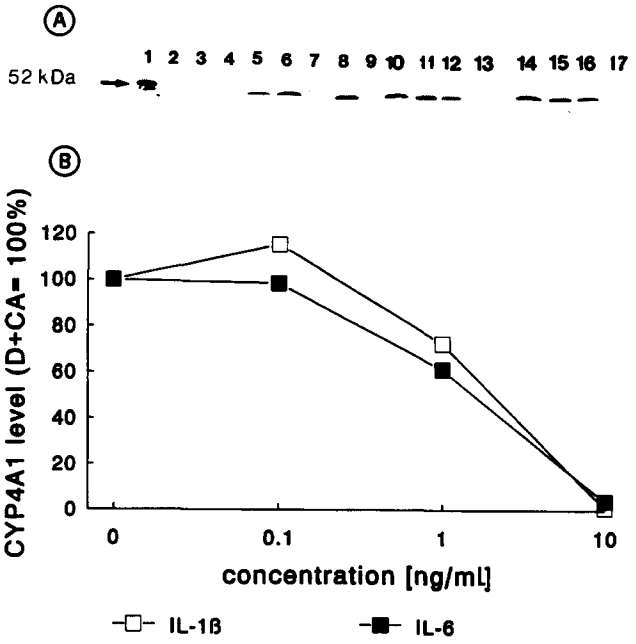

FIG. 3. A, immunoblotting with a polyclonal antirat CYP4A1 antibody of microsomal proteins from cultured fetal rat hepatocytes and rat liver. Cultured cells were treated by inducers and/or interleukins for 72 hr, and homogenates were prepared 96 hr after seeding as described under "Materials and Methods." Rat liver microsomal proteins were used as a control. In each lane, 2 μg of microsomal proteins were applied. The arrow indicates a protein band of M_r 52,000. Lanes 3 to 17 refer to microsomal proteins from cultured cells. Lane 1, clofibrate-induced rat liver microsomal proteins; lane 2, phenobarbital (PB)-induced rat liver microsomal proteins; lane 3, control cells; lane 4, D-treated cells; lane 5, CA-treated cells; lane 6, D + CA-treated cells; lane 7, D + PB-treated cells; lane 8, D + PB + CA-treated cells; lane 9, molecular weight markers; lane 10, D + CA-treated cells; lane 11, D + CA + IL-1β (0.1 ng/mL) -treated cells; lanes 12, D + CA + IL-1β (1 ng/mL) -treated cells; lane 13, D + CA + IL-1β (10 ng/mL) -treated cells; lane 14, D + CA-treated cells; lane 15, D + CA + IL-6 (0.1 ng/mL) -treated cells; lane 16, D + CA + IL-6 (1 ng/mL) -treated cells; lane 17, D + CA + IL-6 (10 ng/mL) -treated cells. B, effects of interleukin concentrations on the CYP4A1 apoprotein content. The densitometric analyses were carried out on six blots. Results are expressed in percentages of the values recorded for cultures induced with dexamethasone plus clofibric acid (arbitrary units, D + CA = 100%).

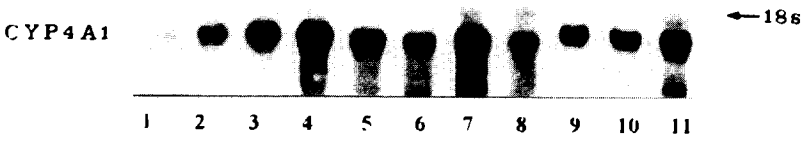

FIG. 4. Northern blot analysis of total RNA from cultured fetal rat hepatocytes with a CYP4A1 cDNA probe. The effects of D, CA, IL-1β, and IL-6 were studied on CYP4A1 mRNA using a specific probe prepared by RT-PCR. The cells were treated with inducers and/or interleukins for 72 hr and harvested by scraping 96 hr after seeding. Northern blots were prepared as described under "Materials and Methods." Filters were rehybridized with a β-actin probe as a control. The CYP4A1, 18 S rRNA, and β-actin signals have relative sizes of 2.0 kb, 2.2 kb, and 2.0 kb, respectively. Results show an experiment representative of three others. Lane 1, control cells; lane 2, D-treated cells; lane 3, CA-treated cells; lane 4, D + CA-treated cells; lane 5, D + CA + IL1β (1 ng/mL) -treated cells; lane 6, D + CA + IL-1β (10 ng/mL) -treated cells; lane 7, D + CA + IL-6 (1 ng/mL) -treated cells; lane, 8, D + CA + IL-6 (10 ng/mL) -treated cells; lane 9, D + IL-1β (10 ng/mL) -treated cells; lane 10, D + IL-6 (10 ng/mL) -treated cells; lane 11, control adult rat liver.

probe was verified by sequencing. It was found suitable for Northern and dot-blotting on fetal rat hepatocyte RNA since its sequence was identical to that of the previously published rPPARα cDNA [43]. The specific rPPARα probe detected two RNA messengers of 2.1 and 1.8 kb, respectively. As previously reported [20], the PPARα transcripts were found to be highly expressed in rat liver (Fig. 5, lane 5, and Fig. 6, lane 12). On the other hand, the total PPARα mRNA level in rat adipose tissue (Fig. 5, lane 6, Table 3) was four times lower than that obtained for control fetal rat hepatocytes (Fig. 5, lane 1, Table 3). Dot-blot analyses revealed that clofibric acid induced a 1.6-fold increase in the PPARα mRNA level of cultured fetal rat hepatocytes (Fig. 5, lane 3; Table 3). The induction was higher (1.9-fold) when the cells were treated with dexamethasone (Fig. 5, lanes 2; Table 3). The combined action of the two inducers resulted in a slight additional increase (Fig. 5, lane 4, Table 3).

As shown by our Northern blot data (Fig. 6; Table 4), the expression of the two PPARα transcripts was induced by clofibric acid (lane 2) and dexamethasone (lane 3), alone or in combination (lane 4). In dexamethasone plus clofibric acid-treated cells, IL-1β (lanes 5-7) as well as IL-6 (lanes 8-10) reduced the induction of the 1.8-kb PPARα mRNA in a dose-dependent manner. The repression exerted by the interleukins was less obvious for the transcript of 2.1 kb. Furthermore, even at the highest concentration used (10 ng/mL), IL-1β (Fig. 6, lane 11) and IL-6 (data not shown) failed to abolish the induction of PPARα by dexamethasone.

TABLE 2. Quantification of CYP4A1 mRNA from Northern blots

	1	2	3	4	5	6	7	8	9	10	11
CYP4A1/actin	27	36	50	70	53	50	65	45	41	42	86

The effects of D, CA, IL-1β, and IL-6 on the level of CYP4A1 mRNA in cultured fetal rat hepatocytes were investigated as described under "Materials and Methods." β-Actin mRNA was used as an internal standard. Results are expressed as the ratio × 10^2 of the signal for CYP4A1 mRNA to that for β-actin mRNA from three experiments. 1, control cells; 2, D-treated cells; 3, CA-treated cells; 4, D + CA-treated cells; 5, D + CA + IL-1β (1 ng/mL) -treated cells; 6, D + CA + IL-1β (10 ng/mL) -treated cells; 7, D + CA + IL-6 (1 ng/mL) -treated cells; 8, D + CA + IL-6 (10 ng/mL) -treated cells; 9, D + IL-1β (10 ng/mL) -treated cells; 10, D + IL-6 (10 ng/mL) -treated cells; 11, adult rat hepatocytes.

DISCUSSION

CYP4A1 Repression by Interleukins

The present study confirms and extends previous reports [24, 25] showing that proinflammatory cytokines inhibit basal and inducible expression of the major CYPs involved in metabolism of xenobiotics. Herein, we have tested the ability of IL-6 to repress the 12-hydroxylation of lauric acid, a specifically CYP4A-related activity, and have observed a

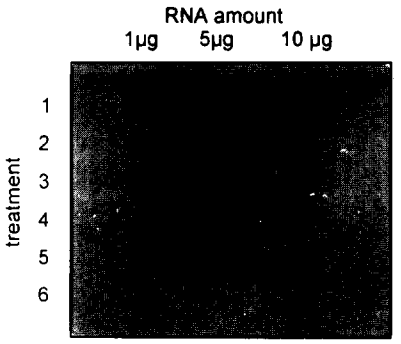

FIG. 5. Dot-blot analysis of total RNA from rat cultured fetal hepatocytes, adult liver, and adipose tissue with a rPPARα cDNA probe. The effects of D and CA were investigated under the conditions described under "Materials and Methods." Results show an experiment representative of three others. Lane 1, control cells; lane 2, D-treated cells; lane 3, CA-treated cells; lane 4, D + CA-treated cells; lane 5, control adult rat liver; lane 6, control adult rat adipose tissue.

TABLE 3. Quantification of PPARα mRNA from dot-blots

Treatment	Slope	Fold change
1. Control cells	3857	1.00
2. Dexamethasone	7211.2	1.87
3. Clofibric acid	6282.5	1.63
4. Dexamethasone + clofibric acid	7864.7	2.04
5. Rat liver	23163	6.00
6. Rat adipose tissue	921	0.24

The effects of D and CA on PPARα mRNA expression were investigated under the conditions described under "Materials and Methods." For each case examined, the slope was calculated as arbitrary units given by the phosphorimagar divided by total RNA in micrograms. Values are normalized to control (untreated) cells and compared with those found for adult rat liver and adipose tissue. Three assays gave similar results.

dose-dependent repressive effect of this interleukin after a 72-hr treatment. The repression exerted by IL-6 was comparable with, although somewhat lesser than, that caused by IL-1β [24]. The time course of this repression was determined for both IL-1β and IL-6. A 9–10-hr treatment was necessary to reduce the induced activity by half. In addition, we have also investigated the effects of IL-1β and IL-6 on the levels of CYP4A1 apoprotein and mRNA. Our results clearly exhibit a reduction in the amount of immunoreactive CYP4A1 protein as the concentration of either interleukin was increased. At a concentration of 10 ng/mL, IL-1β or IL-6 fully suppressed the CYP4A1 induction exerted by the combined action of dexamethasone and clofibric acid. Under the same conditions, the level of the corresponding mRNA was also reduced. These results indicate that a correlation exists between LAH activity and CYP4A1 apoprotein and mRNA levels, except for dexamethasone treatment. It thus appears that both cytokines exert their repressive action at a pretranslational level either by enhancing gene transcription or by stabilizing mRNA. Knickle *et al.* [23] have likewise shown that in rat liver interferon depresses the induced level of CYP4A mRNA and the subsequent synthesis of CYP apoproteins. Pretranslationnal regulation has also been reported for several other CYP isoforms in rat [13, 26] or human [10] hepatocytes. Several hypotheses have been proposed to explain the mechanism of cytokine action. The participation of the nitric oxide pathway, the induction of xanthine oxidase activity, and the increased levels of free radicals or the decrease in heme content have been successively proposed, but these hypotheses have not been proven [5, 10, 44]. Toda *et al.* [45] have recently identified, in the 5′-flanking sequence of the CYP19 gene, a *cis*-acting sequence responsive to the nuclear factor of IL-6 (NF-IL6), a transcription factor activated by IL-1 and IL-6. NF-IL6 enhances the transcriptional activity of the CYP19 gene. The occurrence of such a cytokine-response element in the promoter of CYP4A1 has not yet been reported. On the other hand, CYP4A1 induction is repressed by IL-1 or IL-6, and NF-IL6 enhances the transcription of genes. The overall effect of cytokines on CYP expression cannot be explained by these hypotheses.

TABLE 4. Quantification of PPARα mRNA from Northern blots

	1	2	3	4	5	6	7	8	9	10	11	12
PPARα (2.1 kb)/actin	17	25	32	34	28	30	31	24	29	26	19	43
PPARα (1.8 kb)/actin	15	26	37	38	28	35	42	28	24	18	27	47

The effects of D, CA, IL-1β, and IL-6 on the levels of PPARα transcripts in cultured fetal rat hepatocytes were investigated as described under "Materials and Methods." β-Actin mRNA was used as an internal standard. Results are expressed as the ratio × 10^2 of the signal for PPARα mRNA to that for β-actin mRNA from four independent assays, which gave reproducible results. 1, control cells; 2, Ca-treated cells; 3, D-treated cells; 4, D + CA-treated cells; 5, D + CA + IL-1β (10 ng/mL)-treated cells; 6, D + CA + IL-1β (1 ng/mL)-treated cells; 7, D + CA + IL-β (0.1 ng/mL)-treated cells; 8, D + CA + IL-6 (0.1 ng/mL)-treated cells; 9, D + CA + IL-6 (1 ng/mL)-treated cells; 10, D + CA + IL-6 (10 ng/mL)-treated cell; 11, D + IL-1β (10 ng/mL)-treated cells; 12, adult rat hepatocyptes.

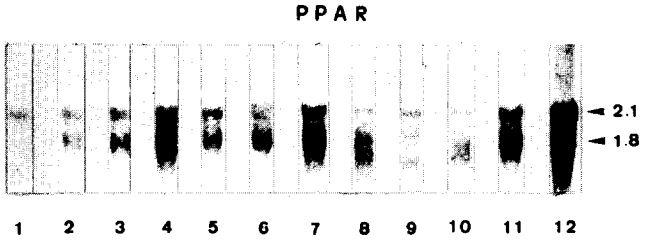

FIG. 6. Northern blot analysis of total RNA from cultured fetal rat hepatocytes with a rPPARα cDNA probe. The effects of D, CA, IL-1β, and IL-6 were studied on PPARα mRNA using a specific probe prepared by RT-PCR. Cells were treated by inducers and/or interleukins for 72 hr and scraped 96 hr after seeding. Northern blots were prepared as described under "Materials and Methods." Filters were rehybridized with β-actin probe as a control. The 18 S rRNA and β-actin signals have relative sizes of 2.2 kb and 2.0 kb, respectively. Results show an experiment representative of four others. Lane 1, control cells; lane 2, CA-treated cells; lane 3, D-treated cells; lane 4, D + CA-treated cells; lane 5, D + CA + IL-1β (10 ng/mL) -treated cells; lane 5, D + CA + IL-1β (1 ng/mL) -treated cells; lane 7, D + CA + IL-1β (0.1 ng/mL) -treated cells; lane 8, D + CA + IL-6 (0.1 ng/mL) -treated cells; lane 11, D + IL-1β (10 ng/mL) -treated cells; lane 12, control adult rat liver.

Induction of PPARα Expression in Cultured Fetal Rat Hepatocytes

It has been recently reported that a specific nuclear receptor, PPARα, mediates CYP4A1 gene transcription by peroxisome proliferators [18]. This receptor binds to a regulatory sequence located 4,300 nucleotides upstream from the rat CYP4A1 gene [18]. This led us to assess PPARα mRNA expression by Northern blotting and dot-blotting using a specific probe. The latter detected two mRNAs of 1.8 and 2.1 kb, respectively. This is in good agreement with recent studies revealing the presence of different specific transcripts for PPARα in rodent liver cells, cultured hepatocytes, and hepatoma cells [21, 38, 46, 47]. In our model, dexamethasone induced PPARα mRNA as previously shown by other authors in adult rat hepatocytes [21, 22]. Clofibric acid also increased the PPARα

mRNA content in cultured fetal rat hepatocytes, but to a lesser extent. The induction exerted by clofibric acid alone or dexamethasone alone was slightly enhanced when their action was combined.

Repression of PPARα Expression

The present study shows that IL-1β or IL-6 represses PPARα expression in cultured fetal rat hepatocytes. Although these cytokines down-regulated the PPARα mRNA level in a dose-dependent manner, they did not abolish the induction exerted by the combined action of clofibric acid and dexamethasone. Insulin, as with interleukins in our model, has also been shown to reduce the PPARα mRNA level in hepatoma cells [21]. The repression of PPARα expression by mediators is thus not restricted to interleukins.

Role of Dexamethasone in the Regulation of CYP4A1 and PPARα

Clofibric acid alone induced LAH activity in cells treated for 72 hr, but the additional presence of dexamethasone doubled the induction factor. Here, we were surprised to find that dexamethasone increased the CYP4A1 mRNA level as compared with control cells. In fact, PPARα is induced in cultured fetal rat hepatocytes by dexamethasone as shown by our results and other studies [21, 22]. The increase in the steady-state CYP4A1 mRNA level occurred after a prerequisite increase in the dexamethasone-induced PPARα mRNA, if we consider that PPARα positively mediates the induction of CYP4A1 in absence of peroxisome proliferators, as shown by Aldridge *et al.* [18]. Such a mechanism might explain the induced level of CYP4A1 mRNA when dexamethasone is added alone. Yet importantly, we observed no parallel increase in the CYP4A1 apoprotein level or enzyme activity under these conditions. On the other hand, clofibric acid added alone concomitantly increases the CYP4A1 mRNA and apoprotein levels as well as the LAH activity (results presented here and in ref. [24]). Whether dexamethasone is applied or not, the presence of clofibric acid is thus required to produce a detectable level of CYP4A1 apoprotein and subsequent LAH activity. The synthesis of a functional CYP4A1 protein by clofibric acid may result from an activation of PPARα by clofibric acid, the activation of a signal transduction pathway distinct from PPARα, or the requirement of different PPARs. However, PPARα is the predominate isotype expressed in the rat liver cells, PPARδ and γ being barely detectable [20].

Protective Effect of Dexamethasone

Our results clearly show that dexamethasone partially prevents CYP4A1 repression due to IL-1β. Furthermore, IL-1β and IL-6 fail to reduce the amounts of CYP4A1 and PPARα mRNA observed in dexamethasone-treated cells. The presence of a glucocorticoid response element upstream from the CYP4A1 gene has not yet been reported. The protective effect of dexamethasone is thus partially caused by the enhanced synthesis of PPARα. In cultured fetal rat hepatocytes, Ferrari *et al.* [14] have also reported a protective effect of dexamethasone against IL-1β repression of the CYP2A and CYP2B subfamilies. The mechanism of the protective effect of glucocorticoids is a subject of debate. In fetal liver, glucocorticoids inhibit the production of cytokines by Kupffer cells [48]. On the other hand, several authors suggest a competition between IL-1β and dexamethasone for binding to the glucocorticoid receptor [49] or a drop in the glucocorticoid receptor population caused by IL-1β or IL-6 [50, 51].

In conclusion, induction of CYP4A1 in fetal rat hepatocytes by the combined action of clofibric acid and dexamethasone is repressed by IL-1β and IL-6 in a dose- and time-dependent manner. The cytokines down-regulate the expression of CYP4A1 at a pretranslational level. This down-regulation may be explained by the fact that these cytokines repress the expression of PPARα, a transcriptional inducer of CYP4A1. Dexamethasone, which enhances PPARα mRNA synthesis, exerts a protective effect against cytokine repression.

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