Rapid Transcriptional Suppression of Rat Cytochrome P450 Genes by Endotoxin Treatment and Its Inhibition by Curcumin

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ABSTRACT

Down-regulation of constitutive hepatic cytochrome P450 (P450) mRNAs by bacterial endotoxin (lipopolysaccharide, LPS) or other inflammatory stimuli has been documented extensively, but the contribution of transcriptional suppression to this effect is poorly understood. Here, we demonstrate that the rates of transcription of the CYP2C11, CYP3A2, and CYP2E1 genes are reduced to 20, 30, and 10% of control levels, respectively, in rat liver within 1 to 2 h of injection of LPS (1 mg/kg). The magnitude and rapidity of these effects indicate that transcriptional suppression is a primary reason for the decline in P450 mRNAs. Injection of curcumin significantly inhibited the rapid transcriptional suppression of CYP2E1, and blocked that of CYP3A2. These effects seemed to be independent of inhibition of nuclear factor-κB (NF-κB) activation by curcumin, because induction of known NF-κB-regulated genes was not attenuated. One hour after LPS injection, the DNA-binding activities of hepatocyte nuclear factor (HNF)1α, HNF3β, and HNF4α were reduced to 73, 72, and 53%, respectively, of control values. The nuclear abundances of Sp1, liver-enriched transcriptional inhibitory protein (LIP), HNF1α, and HNF3β were unchanged, whereas the abundance of HNF4α was reduced to 87% of control levels. We conclude that changes in Sp1 or LIP do not contribute significantly to the early suppression of P450 transcription in the acute phase rat liver. Although changes in DNA-binding activities of HNF1α, HNF3β, and HNF4α are too small individually to explain the observed changes in P450 transcription, the role of each factor in concert with other factors remains to be determined.

The cytochromes P450 (P450s) are a large family of proteins with pivotal roles in the inactivation, as well as in the bioactivation, of drugs and toxins. P450 enzymes also have important physiological roles in steroid biosynthesis, bile acid metabolism, and the generation of bioactive eicosanoids. The expression and activities of cytochrome P450 enzymes in the liver are down-regulated (Morgan, 1997). It is unclear what benefit, if any, this down-regulation confers to the host organism (Morgan, 2001), but it significantly affects the metabolism and clearance of drugs in human and experimental animals (Morgan, 1997).

Administration of bacterial lipopolysaccharide (LPS) to rats is an established model of bacterial infection and sepsis and can mimic the effects of inflammation and infection on P450 expression and activities (Morgan, 1997). After LPS administration, the mRNAs for multiple P450s are suppressed within 6 to 24 h in the livers of rats and mice (Sewer et al., 1996). In contrast, levels of CYP4A mRNAs increase (Sewer et al., 1996). Although the down-regulation of P450 mRNAs is a well characterized phenomenon, only one study has directly demonstrated the suppression of constitutive P450 gene transcription by inflammation. LPS injection caused a suppression of CYP2C11 gene transcription, measured 24 h after injection, to 5% of control levels (Wright and Morgan, 1990). Although the magnitude of this effect could account for the decrease in steady-state hepatic CYP2C11 mRNA levels, it is not known whether transcriptional suppression of CYP2C11 occurs sufficiently rapidly to contribute to the fast decline in CYP2C11 mRNA (Sewer et al., 1996).

Cytokines, such as interleukin-1, interleukin-6, and tumor necrosis factor-α (TNFα), as well as interferon-γ, can mimic the effects of inflammation on P450 expression in vivo and in hepatocyte cultures (Morgan, 1997). Furthermore, mice with targeted deletions of cytokines or cytokine receptors exhibit altered P450 responses to inflammatory stimuli (for review, see Morgan, 2001). These cytokines are therefore considered important physiological roles in steroid biosynthesis, bile acid metabolism, and the generation of bioactive eicosanoids. P450 enzymes also have beneficial, if any, this down-regulation confers to the host organism (Morgan, 2001), but it significantly affects the metabolism and clearance of drugs in human and experimental animals (Morgan, 1997).

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to be important mediators of inflammatory P450 suppression, although there is also evidence that LPS can act directly on hepatocytes (Morgan, 2001).

The roles of specific transcription factors involved in suppression of P450 expression by inflammation are poorly understood. NF-κB is a transcription factor that plays an important role in the inflammatory response. Its activation by diverse inflammatory stimuli is achieved by the phosphorylation of IκB, which binds and sequesters NF-κB in the cytoplasm of unstimulated cells (Ghosh et al., 1998). Phosphorylation of IκB initiates its proteolytic degradation, releasing active NF-κB. A variety of antioxidants have been reported to suppress activation of NF-κB, probably by inhibiting the activation of IκB kinases (Ghosh et al., 1998; Ghosh and Karin, 2002). NF-κB has been implicated in two separate mechanisms of P450 gene regulation. First, NF-κB can directly interact with the aryl hydrocarbon receptor, resulting in attenuation of ligand-induced CYP1A1 transcription through this receptor (Tian et al., 1999). Second, a low-affinity NF-κB binding site exists on the CYP2C11 promoter. Mutation of this site in a CYP2C11 promoter-reporter gene construct abolished the ability of interleukin-1 or LPS to suppress the reporter gene (Iber et al., 2000), suggesting that it acts as a negative regulatory element contributing to CYP2C11 suppression. The role of this element in CYP2C11 suppression in vivo has yet to be determined.

In the present study, we hypothesized that LPS treatment would cause transcriptional suppression of multiple P450 genes in rat liver and that this suppression would occur before the earliest documented time point for P450 mRNA suppression in this model. We further hypothesized that inhibition of NF-κB activation in this model would attenuate the suppression of P450 transcription caused by LPS treatment. To inhibit NF-κB activation in vivo we chose curcumin, a component of turmeric that possesses anti-inflammatory, anti-oxidant, anti-carcinogenic, and free radical scavenging properties (Xu et al., 1997). Many of these effects are consistent with the ability of curcumin to block the activation of NF-κB (Singh and Aggarwal, 1995; Jobin et al., 1999). Last, we examined nuclear levels of several hepatic transcription factors that have been hypothesized to be involved in down-regulation of P450 genes by inflammatory stimuli.

We demonstrate here that LPS treatment causes suppression of CYP2C11, CYP3A2, and CYP2E1 gene transcription in rat liver to 12 to 30% of control levels within 1 to 2 h of injection. This rapid transcriptional suppression of P450 genes was selectively inhibited by curcumin, although apparently not via a mechanism involving NF-κB. The results suggest a possible role of three hepatic transcription factors, HNF1α, HNF3β, and HNF4α, but not of the p20 form of C/EBPβ, in the rapid transcriptional down-regulation of P450 genes.

Materials and Methods

Animals and Treatments. Male Fischer 344 rats (175–200 g) from Charles River Laboratories (Wilmington, MA) were used. They were allowed free access to food and water at all times and were allowed to acclimatize to the animal facility for at least 1 week before use. Escherichia coli LPS, serotype 0127:B8, and curcumin (Sigma-Aldrich, St. Louis, MO) were dissolved in sterile 0.9% saline and injected intraperitoneally. Animals were sacrificed by CO2 asphyxiation. These procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of Emory University.

Nuclear Run-On Assays. Hepatic nuclei were isolated as described by Blobel and Potter (1966). Nuclear run-on assays were performed using a method modified from Wang et al. (1997). Nuclei were incubated in a total volume of 150 μl of 200 mM Tris pH 8, 200 mM MgCl2, 200 mM KCl, 200 mM dithiothreitol, 80 units RNasin (BD PharMingen, San Diego, CA), 2 mM ATP, 2 mM GTP, 2 mM CTP, 0.2 mM UTP, and 200 μCi of [32P]UTP (3,000 Ci/mmol) at 30°C for 20 min. Nuclear RNA was extracted with 800 μl of TRIzol reagent (Invitrogen, Carlsbad, CA) and recovered by ethanol precipitation. Linearized pBS (Stratagene, La Jolla, CA) plasmids containing β-actin, GAPDH, CYP2C11, CYP3A2, CYP2E1, CYP4A1, haptoglobin, and angiotensinogen cDNAs were denatured and applied to a Nyttran filter (Schleicher & Schuell, Keene, NH) using a slot-blot apparatus. The DNA was immobilized by UV irradiation and the filters were prehybridized 2 to 4 h at 42°C in Northern hybridization buffer (Ambion, Austin, TX) with 100 μg/ml yeast tRNA. [32P]RNA was hybridized to the immobilized DNA in the same solution at a concentration of approximately 4 × 106 cpm/ml for 12 to 16 h at 56°C. Blots were washed twice for 5 min at room temperature in low-stringency wash buffer and twice for 20 min at 65°C in high stringency wash buffer (Ambion). The relative amounts of bound radiolabeled mRNA were quantified by phosphorimaging (Amersham Biosciences Inc., Piscataway, NJ), or by exposure to film and densitometry using a Kodak EDAS 290 imaging system (Eastman Kodak, New Haven, CT).

Preparation of Nuclear Extracts and Electrophoretic Mobility Shift Assay (EMSA). Rat liver nuclear extracts were prepared according to Dignam et al. (1983). Briefly, livers (about 8 g) were minced and homogenized in a glass Dounce homogenizer in 20–50 volumes of ice-cold 10 mM potassium phosphate buffer, pH 6.8, containing 1.3 M sucrose, 5 mM MgCl2, 1 mM sodium orthovanadate, and 50 μM sodium molybdate. The homogenate was filtered through four layers of cheesecloth and centrifuged at 1,000g for 15 min. Nuclei were isolated from the resulting pellet by centrifugation through 2.4 M sucrose at 100,000g for 1 h. Nuclear extracts were obtained by homogenizing nuclei in 20 mM Tris- HCl buffer, pH 7.5, containing 0.42 M NaCl, 0.25 M sucrose, 5 mM MgCl2, 20 mM Tris- HCl, pH 7.5, 1 mM sodium orthovanadate, 50 μM sodium molybdate, 0.25 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 0.5 μg/ml aprotinin, (5 μg/ml), and pelleting at 436,000 × g for 4 h in a TLA 100.4 rotor (Optima TLX ultracentrifuge; Beckman Coulter Inc., Fullerton, CA). The resulting clear supernatant was frozen in dry ice and stored at −80°C. The protein concentrations were measured according to the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Double-stranded oligonucleotides were purified by polyacrylamide gel electrophoresis and labeled with polynucleotide kinase and [γ-32P]ATP (6,000 Ci/mmol). Ten micrograms of nuclear extract protein was incubated at 4°C for 1 min with 0.5 μg of poly(dI-dC) and the [32P]-labeled probe (10,000 cpm) in a final volume of 20 μl of 20 mM HEPES, pH 7.9, 80 mM KCl, 5 mM MgCl2, 2% Ficoll, 5% glycerol, 0.1 mM EDTA, and 0.2 mM dithiothreitol. In the case of antibody supershift/inhibition experiments, the incubation conditions were identical except that the indicated amount of antibody was preincubated with nuclear proteins in the presence of binding buffer for 10 min before addition of the other components. The DNA-protein complexes were resolved by electrophoresis on a 4% polyacrylamide/0.5 × Tris borate-EDTA gel and visualized by autoradiography. The oligonucleotide sequences used (together with their complements) were as follows: NF-κB (immunoglobulin κ chain enhancer), 5′-AGTG TGA GGG GAC TTT CCC AGG C-3′; Sp1 (consensus; Santa Cruz Biotechnology Inc., Santa Cruz, CA), 5′-ATT CGA TCG GGG GCC GAC C-3′; HNF1α (rat albumin promoter), 5′-GTG GTC AAT GAT CTA CAG TTA 3′; HNF3β (consensus) 5′-GTT GAC TAA GTC AAT...
Transcriptional Suppression of P450 Genes

AAT CAG AAT CAG-3' and HNF4α (consensus; Santa Cruz Biotechnology), 5'-CTC AGC TTG TAC TTG GGT ACA ACT A-3'.

Reverse Transcriptase-Polymerase Chain Reaction Analysis of Cytokine mRNA Expression. Total liver RNA was prepared by acid-phenol extraction (Chomczynski and Sacchi, 1987). The total RNA concentration was determined spectrophotometrically at 260 nm. Relative levels of cytokine mRNAs in the samples were assayed simultaneously in a multiplex reverse transcriptase-polymerase chain reaction using a commercially available kit (MPCR kit, set 1 for rat inflammatory cytokines; Maxim Biotech, San Francisco, CA) according to the manufacturer’s instructions.

Western Blotting. The relative levels of transcription factors in hepatic nuclear extracts were measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting. Ten micrograms of nuclear proteins was separated on a 7% polyacrylamide gel (NuPAGE Tris-acetate gel; Invitrogen) and transferred electrophoretically (Xcell II blotting apparatus; Invitrogen) at 40 V for 1.5 h onto a nitrocellulose membrane (Schleicher & Schuell). Blots were incubated in PBS containing 0.05% Tween 20 and 5% nonfat dry milk overnight and immunolabeled with the primary antibody and the enhanced chemiluminescence detection system (Amersham Biociences Inc.) according to the manufacturer’s instructions.

The primary antibodies used, and their dilutions, were as follows: C/EBPβ (C-19) 1:1,000; HNF3β (M-20) 1:1,000; HNF4α (C-19) and Sp1 (PEP2) 1:250; NF-κB p50 (NLS) 1:300; NF-κB p65 (C-20) 1:300 (all from Santa Cruz Biotechnology Inc.); and HNF1α 1:250 (BD Biosciences, Bedford, MA). Antigen titrations were performed for each antibody to ensure that the Western blot signals were in the linear range. Signals were quantified by densitometry as described above.

Results

Transcriptional Down-Regulation of P450 Genes. Transcription run-on assays revealed that treatment of rats with 1 mg/kg LPS caused transcriptional suppression of the CYP2C11 and CYP3A2 genes to 17 and 29% of control rates, respectively, within 1 h of injection (Fig. 1; Table 1). Very similar effects were observed 2 h after injection (Fig. 1; Table 1). Transcription of CYP2E1 was significantly suppressed to 12% of control by 2 h after LPS injection. The mean value for CYP2E1 transcription was decreased to a similar extent at the 1-h time point but did not reach statistical significance because of high variability in the control group (Fig. 1, Table 1). Interestingly, transcription of CYP4A1 was significantly increased to 461% of control, 1 h after LPS treatment, but this effect was not detected at 2 h (Fig. 1; Table 1). Transcription of angiotensinogen, an acute phase gene regulated by NF-κB (Ruminy et al., 2001) was increased 6- and 8-fold, respectively, 1 and 2 h after LPS treatment (Fig. 1; Table 1). Transcription of haptoglobin, an acute phase protein regulated primarily via interleukin-6 and signal transduction and activator of transcription protein-3 as well as by C/EBPβ (Wang et al., 2001) was also induced 2–4-fold at these time points (Fig. 1; Table 1). β-Actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene transcription were analyzed for use as controls. As seen from Fig. 1 and Table 1, GAPDH transcription was similar among the different groups, whereas the β-actin signal was fainter and more variable. Therefore, GAPDH transcription was used as the denominator to normalize the results from different experiments.

Effect of Curcumin. We used curcumin, an inhibitor of NF-κB activation (Singh and Aggarwal, 1995), to address the role of this transcription factor in the rapid suppression of P450 gene transcription. Injection of rats with LPS caused a rapid activation of NF-κB that was maximal by 1.5 h after injection and was sustained 3 h after treatment (Fig. 2A). Thereafter, NF-κB DNA binding activity in the nucleus declined to very low levels by 6 h after treatment. Injection of rats with curcumin 1 h before LPS injection produced a dose-dependent inhibition of NF-κB activation (Fig. 2B), with the largest measured effect occurring at the highest dose of curcumin tested, i.e., 1 mg/kg. This dose was chosen for studies on P450 gene transcription. The identity of the major protein complex as NF-κB was verified by supershifting the complex with antibodies to the p65 subunit (Fig. 2C).

The effects of curcumin on the early suppression of P450 gene transcription by LPS treatment are shown in Fig. 1 and Table 1. Curcumin treatment completely prevented the suppression of CYP3A2 by LPS treatment and significantly attenuated the suppression of CYP2E1 transcription from 12% of control to 50% of control. The mean transcription rate of CYP2C11 in nuclei of rats treated with LPS and curcumin was 36% of control, compared with 21% in rats treated with LPS alone, but this difference was not statistically significant. Induction of the acute phase genes by LPS was not affected by curcumin treatment. Curcumin injection alone had no effect on transcription of any of the genes tested.

To determine whether the attenuation of the suppressive effect of LPS on P450 gene transcription by curcumin could be due to inhibition of cytokine release from Kupffer cells, we studied the effect of curcumin on cytokine mRNA levels in livers of LPS-treated rats. As expected, LPS treatment resulted in increased levels of the hepatic mRNAs for TNFα, interleukin-1, and granulocyte colony stimulating factor (Fig. 3). Curcumin treatment did not inhibit the induction of hepatic levels of these cytokine mRNAs; in fact, induction of all three cytokines was slightly but significantly potentiated by curcumin (Fig. 3).
Each reduced significantly to 73, 72, and 53% of control, two hours after a single i.p. dose of 1 mg/kg LPS. B, dose dependence of curcumin inhibition. Rats received the indicated doses of curcumin or an equal volume of dimethyl sulfoxide vehicle 1 h before injection of LPS (1 mg/kg). Rats were killed 2 h after the LPS injection. C, supershift analysis. Nuclear extracts from rats treated with LPS (1 mg/kg) for 3 h were incubated with specific antibodies (Fig. 4). One hour after treatment, the DNA binding activities of HNF1α, HNF3β, and HNF4α were each reduced significantly to 73, 72, and 53% of control, respectively, in the livers of LPS-injected rats (Fig. 4; Table 2). Only in the case of HNF4α was the decrease in binding activity accompanied by a significant decrease (to 87% of control) in nuclear protein abundance (Fig. 5; Table 2). In contrast, the DNA binding activity and nuclear abundance of another basal factor, Sp1, were not significantly affected by LPS treatment (Figs. 4 and 5; Table 2). Two proteins were detected in rat liver using the antibody to HNF3β. The larger of these bands is identified as HNF3β, based on the measured molecular mass of 54 kDa and comigration with a single band in HepG2 cell extracts (data not shown) and is unaffected by LPS treatment (Fig. 5; Table 2). Interestingly, the smaller, unidentified 49-kDa protein was decreased to 50% of control levels by LPS treatment.

We also measured the effect of LPS treatment on nuclear abundance of the p34, p38, and p20 (LIP) forms of C/EBPβ in rat liver, because nuclear induction of LIP has been implicated in the down-regulation of CYP3A4 by interleukin-6 (Jover et al., 2002). No significant effect on nuclear abundance of these three forms of C/EBPβ was found (Fig. 5; Table 2). As expected, the nuclear levels of the p50 and p65 subunits of NF-κB were markedly increased 1 h after LPS treatment (Fig. 5; Table 2), in agreement with the EMSA data (Fig. 2).

Discussion

Our results show for the first time that suppression of the transcription of multiple constitutive P450 genes after LPS administration is a rapid event, occurring within 1 h of LPS activation. The magnitudes of these early events are similar to the magnitudes by which the CYP2C11, CYP3A2, and CYP2E1 mRNAs are down-regulated at 6 to 12 h after injection (Sewer et al., 1996). Together, these observations demonstrate that inhibition of transcription is a primary mechanism by which down-regulation of P450 expression by inflammatory stimuli is achieved. It is probable that increased rates of mRNA degradation also contribute to P450 mRNA down-regulation, because the levels of these mRNAs have almost reached their respective nadirs only 6 h after LPS injection (Sewer et al., 1996), whereas the measured half-lives of CYP2C11 mRNA in primary rat hepatocytes and of CYP2E1 mRNA in HepG2 cells are 9.8 and 9.6 h, respectively (Peng and Coon, 1998; Iber et al., 2001).

Hepatic CYP4A mRNAs are induced in livers of rats treated with endotoxin (Sewer et al., 1996). Interestingly, we found that CYP4A1 transcription was induced within 1 h, but

TABLE 1
Effects of LPS and curcumin treatment on hepatic P450 and acute phase gene transcription rates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CYP2C11</th>
<th>CYP3A2</th>
<th>CYP2E1</th>
<th>CYP4A1</th>
<th>Haptoglobin</th>
<th>Angiotensinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1 h)</td>
<td>100 ± 10</td>
<td>100 ± 9</td>
<td>100 ± 45</td>
<td>100 ± 10</td>
<td>100 ± 16</td>
<td>100 ± 74</td>
</tr>
<tr>
<td>LPS (1 h)</td>
<td>17 ± 4*</td>
<td>29 ± 8*</td>
<td>10 ± 6</td>
<td>461 ± 49</td>
<td>375 ± 62</td>
<td>627 ± 213*</td>
</tr>
<tr>
<td>Control (2 h)</td>
<td>100 ± 15</td>
<td>100 ± 21</td>
<td>100 ± 29</td>
<td>100 ± 17</td>
<td>100 ± 12</td>
<td>100 ± 32</td>
</tr>
<tr>
<td>Curcumin (2 h)</td>
<td>106 ± 12</td>
<td>107 ± 14</td>
<td>95 ± 7</td>
<td>65 ± 12</td>
<td>117 ± 13</td>
<td>96 ± 25</td>
</tr>
<tr>
<td>LPS (2 h)</td>
<td>21 ± 3*</td>
<td>30 ± 8*</td>
<td>12 ± 3*</td>
<td>72 ± 12</td>
<td>196 ± 17</td>
<td>842 ± 217*</td>
</tr>
<tr>
<td>LPS + curcumin (2 h)</td>
<td>36 ± 10*</td>
<td>108 ± 23*</td>
<td>50 ± 12*</td>
<td>139 ± 25*</td>
<td>200 ± 14*</td>
<td>1011 ± 250*</td>
</tr>
</tbody>
</table>

* Significantly different from control, p < 0.05.
* Significantly different from LPS-treated group (shown for LPS + curcumin group only), p < 0.05.
Our laboratory previously reported the existence of a low-affinity NF-κB binding site at the CYP2C11 promoter, whose mutation abolished the suppression of a CYP2C11 reporter gene in primary hepatocytes (Iber et al., 2000). To determine whether this element is important for CYP2C11 suppression in vivo, we administered curcumin to rats, which effectively blocks NF-κB activation in a variety of cell types (Singh and Aggarwal, 1995) and in vivo (Thaloor et al., 1999). However, the degree of inhibition of NF-κB activation caused by curcumin treatment in this study (Fig. 2) was insufficient to block the early induction of genes known to be NF-κB-dependent, including angiotensinogen (Table 1), interleukin-1, and TNFα (Fig. 3). It is possible that suppression of CYP2C11 by NF-κB via its low-affinity binding site might be more sensitive to partial inhibition of NF-κB. In support of this idea, we observed a tendency toward reversal of LPS suppression of CYP2C11 transcription by curcumin, but the effect was not significant. Studies with more specific and efficacious inhibitors of NF-κB are necessary to establish or exclude its role in CYP2C11 down-regulation.

Participation of NF-κB in regulation of the CYP3A2 and CYP2E1 genes has not been reported. Therefore, our discovery that the rapid suppression of these genes is attenuated (or blocked completely in the case of CYP3A2) was unexpected. The mechanism by which curcumin achieves these effects is unknown. Curcumin has diverse effects on cellular functions including inhibition of c-Jun kinase and activator protein-1 activation (Chen and Tan, 1998), inhibition of tyrosine kinases and protein kinase C (Chen and Huang, 1998), and scavenging of superoxide, hydroxyl, and peroxy radicals (Gerhauser et al., 2003) that could potentially contribute to the observed effects. However, the effects of curcumin on the rapid suppression of P450 transcription are probably not due to inhibition of cytokine release from nonparenchymal cells, because it did not inhibit LPS induction of cytokine mRNAs at this early time point. Curcumin may be a useful tool to study mechanisms of transcriptional suppression of CYP3A2 and CYP2E1.

Several potential mechanisms of transcriptional P450 suppression have been suggested by previous work on regulation of P450 reporter gene constructs and on other genes that are suppressed during the hepatic acute phase response. Therefore, we examined the effect of LPS treatment on levels and/or DNA binding activity of transcription factors previously implicated in these mechanisms. We emphasize that the following discussion pertains only to the rapid suppression of P450 transcription. It is possible that different mechanisms of suppression are important for P450 suppression during different phases of the response, as is true for regulation of acute phase genes (Ruminy et al., 2001).

C/EBPβ is a transcription factor whose expression and DNA binding activity is increased in acute phase liver (Ruminy et al., 2001). Use of alternative initiation codons in the C/EBPβ mRNA can result in the generation of a 20-kDa truncated form (LIP) that lacks the transactivation domain. LIP antagonizes the transcriptional activity of full-length C/EBPβ and C/EBPα. Hepatic nuclear levels of LIP are increased in the livers of rats within 3 h of LPS injection (An et al., 1996). Jover et al. (2002) recently demonstrated that expression of LIP represses human CYP3A4 reporter gene expression, suggesting that LIP induction mediates the suppression of CYP3A4 in acute phase livers. Here, we found that levels of LIP were not significantly affected in rat liver not at 2 h after LPS treatment. This transient induction of CYP4A1 transcription is unlikely to explain the sustained increase in CYP4A1 mRNA caused by LPS injection, and more work is needed to determine when the presumed second phase of induced CYP4A1 transcription occurs.

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C/EBPβ is a transcription factor whose expression and DNA binding activity is increased in acute phase liver (Ruminy et al., 2001). Use of alternative initiation codons in the C/EBPβ mRNA can result in the generation of a 20-kDa truncated form (LIP) that lacks the transactivation domain. LIP antagonizes the transcriptional activity of full-length C/EBPβ and C/EBPα. Hepatic nuclear levels of LIP are increased in the livers of rats within 3 h of LPS injection (An et al., 1996). Jover et al. (2002) recently demonstrated that expression of LIP represses human CYP3A4 reporter gene expression, suggesting that LIP induction mediates the suppression of CYP3A4 in acute phase livers. Here, we found that levels of LIP were not significantly affected in rat liver not at 2 h after LPS treatment. This transient induction of CYP4A1 transcription is unlikely to explain the sustained increase in CYP4A1 mRNA caused by LPS injection, and more work is needed to determine when the presumed second phase of induced CYP4A1 transcription occurs.

Our laboratory previously reported the existence of a low-affinity NF-κB binding site at the CYP2C11 promoter, whose mutation abolished the suppression of a CYP2C11 reporter gene in primary hepatocytes (Iber et al., 2000). To determine whether this element is important for CYP2C11 suppression in vivo, we administered curcumin to rats, which effectively blocks NF-κB activation in a variety of cell types (Singh and Aggarwal, 1995) and in vivo (Thaloor et al., 1999). However, the degree of inhibition of NF-κB activation caused by curcumin treatment in this study (Fig. 2) was insufficient to block the early induction of genes known to be NF-κB-dependent, including angiotensinogen (Table 1), interleukin-1, and TNFα (Fig. 3). It is possible that suppression of CYP2C11 by NF-κB via its low-affinity binding site might be more sensitive to partial inhibition of NF-κB. In support of this idea, we observed a tendency toward reversal of LPS suppression of CYP2C11 transcription by curcumin, but the effect was not significant. Studies with more specific and efficacious inhibitors of NF-κB are necessary to establish or exclude its role in CYP2C11 down-regulation.

Participation of NF-κB in regulation of the CYP3A2 and CYP2E1 genes has not been reported. Therefore, our discovery that the rapid suppression of these genes is attenuated (or blocked completely in the case of CYP3A2) was unexpected. The mechanism by which curcumin achieves these effects is unknown. Curcumin has diverse effects on cellular functions including inhibition of c-Jun kinase and activator protein-1 activation (Chen and Tan, 1998), inhibition of tyrosine kinases and protein kinase C (Chen and Huang, 1998), and scavenging of superoxide, hydroxyl, and peroxy radicals (Gerhauser et al., 2003) that could potentially contribute to the observed effects. However, the effects of curcumin on the rapid suppression of P450 transcription are probably not due to inhibition of cytokine release from nonparenchymal cells, because it did not inhibit LPS induction of cytokine mRNAs at this early time point. Curcumin may be a useful tool to study mechanisms of transcriptional suppression of CYP3A2 and CYP2E1.

Several potential mechanisms of transcriptional P450 suppression have been suggested by previous work on regulation of P450 reporter gene constructs and on other genes that are suppressed during the hepatic acute phase response. Therefore, we examined the effect of LPS treatment on levels and/or DNA binding activity of transcription factors previously implicated in these mechanisms. We emphasize that the following discussion pertains only to the rapid suppression of P450 transcription. It is possible that different mechanisms of suppression are important for P450 suppression during different phases of the response, as is true for regulation of acute phase genes (Ruminy et al., 2001).

C/EBPβ is a transcription factor whose expression and DNA binding activity is increased in acute phase liver (Ruminy et al., 2001). Use of alternative initiation codons in the C/EBPβ mRNA can result in the generation of a 20-kDa truncated form (LIP) that lacks the transactivation domain. LIP antagonizes the transcriptional activity of full-length C/EBPβ and C/EBPα. Hepatic nuclear levels of LIP are increased in the livers of rats within 3 h of LPS injection (An et al., 1996). Jover et al. (2002) recently demonstrated that expression of LIP represses human CYP3A4 reporter gene expression, suggesting that LIP induction mediates the suppression of CYP3A4 in acute phase livers. Here, we found that levels of LIP were not significantly affected in rat liver
nuclei 1 h after LPS injection, and therefore we conclude that suppression of rat liver CYP3A2, 2C11, and 2E1 transcription at early time points is not due to elevation of nuclear LIP. The physiological importance of LIP is presently unclear, because recent studies have shown that LIP can be generated in nuclear extracts by artifactual proteolysis (Baer and Johnson, 2000).

We then studied three transcription factors that are involved in the basal transcription of CYP2C11, CYP3A2, and/or CYP2E1. HNF1α down-regulation by inflammation is causative for the down-regulation of several genes, including albumin, α1-microglobulin (Ruminy et al., 2001), CYP27A (Memon et al., 2001), and the sodium-dependent bile acid transporter ntcp (Trauner et al., 1998). HNF1α transactivates the promoters of both CYP2E1 (McGehee et al., 1997) and CYP2C11 (Park and Waxman, 2001). Roe et al. (2001) reported that HNF1 DNA-binding activity in hepatic nuclei is greatly reduced only 1 h after LPS injection and suggested that this change was causative for CYP2E1 suppression. HNF3β plays an important role in the basal transcription of CYP2C11 (Park and Waxman, 2001) as well as of other CYP2C genes. Decreased expression of HNF3β is implicated in altered gene regulation during an acute phase response (Ruminy et al., 2001). HNF4α is essential for basal transcription of rat CYP3A2 and CYP3A1 reporter gene constructs (Ogino et al., 1999) and for inducible expression of human CYP3A4 (Tirona et al., 2003). We found here that the DNA-binding activities of HNF1α, HNF3β, and HNF4α were significantly reduced in livers of LPS-treated rats, with HNF4α exhibiting the largest reduction to 53% of control. The magnitudes of the observed effects suggest that, although the reduced activities of these factors could each contribute to the observed rapid transcriptional suppression of P450 genes, the suppression is likely to be caused by a combination of changes in activities of important basal transcription factors such as the HNFs, as well as by the activation of negative regulatory factors, e.g., NF-κB.

Stat5b is a transcription factor that is essential for the gender-dependent expression of many P450 genes. However, we were unable to discern whether LPS had an effect on Stat5b, due to the well characterized cyclic physiological variation in its DNA-binding activity (data not shown). Peng and Coon (2000) found an Sp1 binding site in the rabbit CYP2E1 gene that was required for induction of CYP2E1 reporter gene expression by interleukin-1α. However, we found no significant change in nuclear Sp1 levels or DNA binding activity 1 h after LPS treatment. Therefore, Sp1 is not responsible for the observed changes in CYP2E1, 2C11, or 3A2 transcription.

The observed changes in nuclear HNF1α and HNF3β DNA-binding activity were not accompanied by changes in their nuclear protein abundances. Similarly, the 47% reduction in and HNF4α DNA-binding activity is compared with only a small (13%) decrease in nuclear abundance. Therefore, consistent with the rapidity of the effects, the dominant mechanisms for these reductions in HNF activities are likely to be via post-translational modification. Interestingly, the
DNA-binding activity of HNF4α was reported to be reduced by nitric oxide (Hara and Adachi, 2002). However, we have shown that down-regulation of CYP 2C11, 2E1, and 3A2 mRNAs are independently of nitric oxide synthesis (Sewer and Morgan, 1998). On the other hand, there is evidence that phosphorylation state can regulate the transcriptional activity of HNF1α (Guo et al., 2003) and of hepatocyte HNF4α in response to interleukin-1 (Carriere et al., 2001). Little is known about the post-translational regulation of HNF3β.

In summary, we have demonstrated that transcription of constitutive P450 genes 2C11, 3A2, and 2E1 is rapidly suppressed in the livers of rats treated with endotoxin. The rapid suppression of CYP3A2 and CYP2E1 is attenuated by curcumin via an unknown mechanism. Suppression of the three P450 genes is not due to increased nuclear levels of LIP or to decreased nuclear activity of Sp1. Concomitant with transcriptional suppression, DNA-binding activities of HNF1α, HNF3β, and HNF4α are each decreased in rat liver, mainly by post-translational mechanisms. Therefore, the possible roles of HNF1α, HNF3β, and HNF4α in the rapid suppression of hepatic P450 genes by LPS deserve further study.

Acknowledgments

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**Treatment**

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**Antigen**

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<tr>
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**References**


Fig. 5. Effects of LPS treatment on nuclear transcription factor levels in rat liver. Groups of four rats were injected i.p. with saline or 1 mg/kg LPS, and their livers were harvested 1 h later for preparation of nuclear extracts. Relative levels of the indicated transcription factors were determined by Western blotting as described in the text. Each lane represents an individual rat liver. The indicated apparent molecular masses of the proteins detected by the C/EBPβ antibody are from An et al. (1996). The values measured in our laboratory were 40, 33, 29, and 18 kDa, respectively.


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