Bile flow is rapidly and markedly reduced in hepatic inflammation, correlating with suppression of critical hepatic bile acid transporter gene expression, including the principal hepatic bile acid importer, the Na+/taurocholate co-transporting polypeptide (Ntcp, Slc10a1). Endotoxin treatment of rats and interleukin-1β (IL-1β) treatment of liver-derived HepG2 cells leads to a marked decline in the nuclear binding activity of a main Ntcp gene regulator, the nuclear receptor heterodimer retinoid X receptor:retinoic acid receptor (RXR:RAR). How IL-1β signaling leads to reduced RXR:RAR nuclear binding activity is unknown, and we sought to determine whether mitogen-activated protein kinase (MAPK) pathways were involved. IL-1β treatment of cultured primary rat hepatocytes markedly reduced Ntcp RNA levels and Ntcp promoter activity in transiently transfected HepG2 cells. Pretreatment with inhibitors of extracellular signal-regulated kinase (ERK, PD98059) or p38 MAPK (SB203580) did not affect IL-1β-mediated suppression of Ntcp gene expression, whereas curcumin, a derivative of the spice turmeric and a recently described inhibitor of c-Jun N-terminal kinase (JNK), completely ameliorated the effects of IL-1β. Co-transfection of a JNK expression plasmid inhibited RXR:RAR-mediated activation of the Ntcp promoter, while a dominant negative JNK expression plasmid completely blocked IL-1β-mediated suppression. Curcumin, but not PD98059 or SB203580, inhibited IL-1β-mediated suppression of nuclear RXR:RAR binding activity, which correlated with inhibition of JNK phosphorylation and phospho-JNK-mediated phosphorylation of RXR. Taken together, these data provide evidence supporting a novel player (JNK), as well as its inhibitor (curcumin), in inflammation-mediated regulation of hepatobiliary transporters and correlate JNK-dependent RXR phosphorylation with reduced RXR-dependent hepatic gene expression.

Bile flow is markedly impaired in a variety of inflammatory conditions. In animal models of inflammation, including systemic infection, endotoxin, and direct administration of cytokines to rodents and isolated hepatocytes, the generation of bile is significantly and reproducibly reduced to low levels leading to cholestasis (1–6). Recent findings (see reviews in Refs. 5–7) indicate that inflammation-mediated down-regulation of bile flow is because of a complex and coordinated reduction in the expression and function of critical resident hepatic membrane transporters at both transcriptional and post-transcriptional levels. Little is known of the underlying cellular and molecular mechanisms, but several groups have focused their efforts by trying to link transporter gene down-regulation to the various arms of the intracellular signaling mechanisms invoked during the hepatic response to inflammation (the acute phase response).

The expression and function of hepatic bile acid transporters have been studied in a variety of experimental inflammatory conditions, with the main emphasis on alterations in bile acid uptake and Ntcp gene regulation (reviewed in Ref. 8). The hepatocyte responds to inflammation and cholestasis by a variety of self-protective adaptations, including reduction in the sinusoidal uptake of bile acids. We and others (1, 3, 4, 9) have found that endotoxin treatment of animals, or cytokine administration to cells, leads to down-regulation of Ntcp transport function, protein expression, mRNA levels, transcription initiation, and promoter activity. This inflammation-induced repression of Ntcp gene expression is due to reduced nuclear concentrations of key Ntcp promoter transcriptional activators, primarily hepatocyte nuclear factor 1 (HNF1) and the nuclear receptor heterodimer retinoid X receptor:retinoic acid receptor (RXR:RAR) (4, 9). In human hepatoblastoma-derived HepG2 cells, interleukin-1β (IL-1β) down-regulates Ntcp promoter activity by reducing RXR:RAR function and DNA binding activity (9). Moreover, we have recently shown that bile acids also down-regulate the Ntcp promoter via repression of RXR:RAR function by the bile acid-induced expression of the transcriptional repressor small heterodimer partner (SHP, NR0B2) (10). Together, these findings support the concept that RXR:RAR is a central mediator of Ntcp gene activity and that both cholestatic and cytokine-activated pathways regulate Ntcp gene expression via distinct, and likely additive, suppression of RXR:RAR function.

Whether or not there is a direct link between cytokine- and

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The abbreviations used are: RXR, retinoid X receptor; RAR, retinoic acid receptor; SHP, small heterodimer partner; IL-1β, interleukin-1 β; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; dnJNK, dominant negative JNK; MAPK, mitogen-activated protein kinase; GST, glutathione S-transferase; AP-1, activator protein 1; CYP7A1, cholesterol 7α-hydroxylase; DR2, direct repeat 2; TK, herpes simplex virus thymidine kinase; Me 2SO, dimethyl sulfoxide; wt, wild type; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; pNPP, p-nitrophenyl phosphate.

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bile acid-regulated pathways in liver is controversial and currently under investigation. Gupta et al. (11) provide support for bile acid activation of the SHP promoter via a mechanism that involves a central player in the response to inflammation, c-Jun N-terminal kinase, JNK. However, a role for SHP in the endotoxin-mediated down-regulation of the Ntcp gene is not supported by a recent publication of Zollner et al. (12), where Ntcp expression was clearly reduced in response to both bile acid feeding and bile duct ligation (conditions that lead to increased SHP expression), whereas endotoxin administration reduced Ntcp RNA levels without enhancing SHP RNA expression. Thus, the role of SHP in mediating inflammation-mediated down-regulation of the Ntcp gene is unclear. In this report, we present evidence that IL-1β-mediated Ntcp promoter down-regulation does not necessarily require SHP; rather, IL-1β treatment leads to JNK-dependent repression of RXR:RAR nuclear binding activity with consequent suppression of RXR:RAR-mediated transcription. Moreover, we employ curcumin, a component of the spice turmeric, as a JNK inhibitor that completely abrogates Ntcp gene promoter suppression by IL-1β (13). These studies provide support for a novel and potentially widely targeted pathway of gene regulation in liver—direct JNK-dependent phosphorylation of RXR.

EXPERIMENTAL PROCEDURES

Materials and Plasmids—IL-1β was purchased from R&D Systems Inc. (Minneapolis, MN) and curcumin, PD98059, and SB203580 from Calbiochem. Routine research reagents were purchased from Sigma. Plasmids containing wild type (wt) minimal rat Ntcp promoter (nucleotide –158/+47) inserted into the luciferase vector pVOSaLΔ5', as well as the FM1 plasmid containing mutations in the Ntcp RXR:RAR binding site (Direct Repeat 2 (DR2) element wt –53GGGCA-TAAAGTTA –46, FM1 –53GGCAGTAAGTGGA –46) where hexamer binding sites are underlined and mutated nucleotides are in lowercase) were constructed as previously described (9, 14). Isolated wt and FM1 Ntcp DR2 elements were inserted upstream of the herpes simplex virus thymidine kinase promoter (TK) as previously described (9). Plasmids expressing active JNK1 and dominant negative JNK1 (dnJNK) were generous gifts from Drs. James Woodgett (Ontario Cancer Institute, Toronto, CA) and Bing Su (M.D. Anderson Cancer Center, Houston, TX), respectively. Rat Ntcp and Gapdh probes and reagents were used as described previously (4).

Primary Rat Hepatocytes and HepG2 Cell Cultures—Primary rat hepatocytes were obtained from male Sprague-Dawley rats via a modification of the collagenase perfusion method of Berry and Friend (10, 16). Five million hepatocytes purified through a Percoll gradient were used as described previously (4).

Cell Treatments—Four hours after plating, primary rat hepatocytes in serum-free medium were exposed to inhibitors (25 μm SB203580) or equal volume of Me 2SO vehicle) 30 min prior to the addition of 1 ng/ml IL-1β (or saline control) for the total treatment time of 16 h. HepG2 cells were subjected to treatments with either 1 ng/ml IL-1β or water control for time periods varying from 5 min to 16 h before harvest.

RESULTS

IL-1β-mediated Down-regulation of Ntcp Expression Is Curcumin-sensitive—We first sought to determine whether IL-1β treatment of primary rat hepatocyte cultures leads to reductions in Ntcp RNA levels and whether known inhibitors of IL-1β signaling affect the response of hepatocytes to IL-1β (Fig. LA). Treatment with 1 ng/ml of IL-1β for 16 h reduced Ntcp RNA levels by 70% compared with saline control, thereby repriming assays with reporter lysis buffer (Promega Corp., Madison, WI) according to the manufacturer’s instructions. Luciferase activity was determined via an Ascent microplate luminometer (Thermo Labsystems, Helsinki, Finland) and normalized to β-galactosidase activity as previously described (14). Each transfection experiment was performed in triplicate, repeated 3–6 times, and validated using at least 2 different plasmid preparations.

RNA Analysis—Total RNA was extracted from plated primary rat hepatocytes by guanidium thiocyanate extraction with subsequent centrifugation in cesium chloride solution (10). 30 μg of total RNA was electrophoresed through 1% denaturing agarose gels as described. After transfer to nylon membranes, blots were incubated with 32P-labeled cDNA probes for rat Ntcp or GAPDH, washed, and exposed to Kodak BioMax film according to standard procedures (10). RNA quantitation was performed with a PhosphorImage (Amersham Biosciences).

Electrophoretic Mobility Shift Assays—Crude nuclear extract was prepared from treated HepG2 cells according to published methods (18). Protein concentrations were determined using the Bradford reagent kit (Bio-Rad). Double-stranded wt Ntcp RXR:RAR DR2 element (nucleotide –53/–40) was end-labeled, purified, and incubated with 10 μg of HepG2 nuclear extracts for 30 min as described (4). After binding, each reaction was electrophoresed on a non-denaturing 5% polyacrylamide gel, dried, and exposed to BioMax film for varying time periods. The canonical AP-1 element-containing oligonucleotide 5¢-CAGCTGATG-GAG-3¢ was tested in a similar fashion.

Immunoblotting—Total cell extracts were obtained by homogenizing in 0.25 ml of buffer (50 mM Tris-HCl, pH 7.5, 0.5 mM CaCl2, 2 mM EDTA, 2 mM EGTA, 1% Triton X-100, 0.25% deoxycholate, 1 μg/ml phosphatase, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml Na3VO4), and protein levels were determined by the BCA protein assay method according to the manufacturer’s instructions (Pierce). Protein samples were resolved by 10% SDS-PAGE and transferred to Trans-Blot transfer membrane (Bio-Rad) at 250 mA for 75 min. Membranes were blocked (1 h at room temperature) in 5% nonfat dry milk dissolved in TBST (Tris-buffered saline Tween) prior to incubation with antibodies specific for phospho-JNK or JNK (both 1:2000; Cell Signaling Technology, Beverly, MA) for 16 h at 4 °C. Antibodies were diluted in 5% bovine serum albumin in TBST. Membranes were subsequently washed three times with TBST and incubated with secondary antibody (anti-rabbit goat IgG linked with horseradish peroxidase, 1:2000 in 5% milk in TBST) for 1 h at room temperature. After washing in TBST three times, blots were incubated with ECL reagents for 1 min according to the manufacturer’s instructions (Western lighting chemiluminescent reagent plus, PerkinElmer Life Sciences).

Protein Kinase Assays—Glutathione S-transferase (GST)-tagged RXR constructs were expressed in Escherichia coli BL21 (Stratagene, La Jolla, CA) and purified through glutathione-Sepharose beads according to the manufacturer’s recommendations (Amersham Biosciences) (19). Immune complex kinase assays were performed as previously described (19, 20) to examine JNK activity. Briefly, HepG2 cells were grown to confluency, placed in serum-free media for 48 h, and treated for the indicated time periods with 1 ng/ml IL-1β, ± inhibitors curcumin, PD98059, or SB203580 or vehicle MeSO. Cells were lysed and JNK immunoprecipitated from 100 μg of cell extract with antibodies (1 μg) that recognize JNK (Santa Cruz Biotechnology, Santa Cruz, CA). Protein A-G agarose beads (20 μl) (Santa Cruz Biotechnology) were added and incubated at 4 °C for 1 h. Beads were washed three times with lysis buffer. Ten μl of kinase buffer (20 μM Hepes, pH 7.5, 20 μM β-glycerol phosphate, 10 mM NaN3, 1 mM dithiothreitol, and 50 μM sodium vanadate). Kinase assays were performed by incubating the beads with 30 μl of kinase buffer to which 20 μM cold ATP, 5 μCi of [γ32P]ATP (2000 cpm/pmol), and 2 μg of GST-RXR were added. The kinase reaction was performed at 30 °C for 20 min. The samples were suspended in Laemmli buffer, boiled for 5 min, and analyzed by SDS-PAGE. The gel was dried and autoradiographed.
producing the known in vivo effects of endotoxin and cytokines (3, 4). IL-1β regulates the activity of a variety of target genes and transcription factors through several signal transduction cascades, including the three main mitogen-activated protein kinases (MAPK), which are JNK, extracellular signal-regulated kinase (ERK), and p38 MAPK (21, 22). To investigate which pathway or pathways were primarily involved, primary rat hepatocytes were preincubated with known inhibitors of these three signaling pathways immediately prior to exposure to IL-1β (Fig. 1A). Preincubation with a known ERK (20 μM PD98059) or p38 MAPK (25 μM SB203580) inhibitor had no significant effect on IL-1β-mediated down-regulation of Ntcp RNA levels, whereas curcumin, a recently described JNK inhibitor, completely blocked the effects of IL-1β (13).

We have recently shown (9) that IL-1β treatment of HepG2 cells inhibits rat Ntcp promoter activity through reduction in the nuclear binding activity of the trans-acting nuclear receptor heterodimer RXR:RAR. In order to explore the molecular mechanisms underlying IL-1β-mediated suppression of Ntcp promoter activity, it was first necessary to determine whether the response to the three inhibitors employed in IL-1β-treated primary rat hepatocyte experiments was replicated in transiently transfected HepG2 cells. As previously reported (9), treatment of HepG2 cells transiently transfected with wt -158/+47 Ntcp promoter-luciferase constructs with IL-1β resulted in suppression of reporter activity by ~50% (Fig. 1B). Pretreatment with the JNK inhibitor, curcumin, completely blocked IL-1β-mediated repression of Ntcp promoter activity, whereas treatment with either the ERK (PD98059) or the p38 MAPK (SB203580) inhibitors had no discernible effect. These data validate the use of transiently transfected HepG2 cells as a model of curcumin inhibition of the effects of IL-1β on Ntcp gene expression.

**IL-1β Suppression of the RXR:RAR Element in the Ntcp Promoter Is JNK-dependent**—One of the principal mechanisms of action attributed to the effects of curcumin on inflammation-based signal transduction is via inhibition of JNK (23). Although it is clear from previous work that an intact DR2 RXR:RAR binding element in the Ntcp promoter is required for IL-1β repression, a role for JNK in regulating Ntcp promoter expression is unknown (9). In order to support a role for JNK in IL-1β-mediated suppression of RXR:RAR transactivation of the Ntcp promoter, it was necessary to determine whether 1) JNK can directly suppress RXR:RAR-dependent activation of the Ntcp promoter, 2) the effects of IL-1β can be blocked by interfering with JNK signaling by co-transfecting a plasmid expressing a dominant negative version of JNK (dnJNK), and 3) RXR:RAR binding to the DR2 element in the Ntcp promoter is necessary for the effects of IL-1β and JNK. As seen in Fig. 2A, IL-1β suppresses expression of the wt Ntcp promoter but not...
IL-1β Suppression of the Ntcp Promoter via JNK

The RXR:RAR binding site (DR2 element) is functionally mutated (FM1). Co-transfection of a plasmid expressing an active form of JNK1 (JNK) significantly suppresses Ntcp promoter activity, with further suppression in the presence of IL-1β/H9252. Notably, co-transfection of the dnJNK plasmid completely abrogates the effects of IL-1β/H9252. The expression of the mutant Ntcp promoter plasmid FM1 was less than that of the wt Ntcp promoter-luciferase plasmid (as previously reported) and was insensitive to the effects of IL-1β/H9252 and co-transfected plasmids JNK and dnJNK. Thus both IL-1β- and JNK-mediated down-regulation of the Ntcp promoter requires an intact DR2 element, and co-transfection of a dnJNK plasmid completely interferes with IL-1β suppression of the Ntcp promoter.

The data in Fig. 2A show that mutation of the DR2 element completely eliminated the effects of IL-1β as well as co-transfected JNK and dnJNK plasmids on Ntcp promoter expression, which could reflect effects on neighboring Ntcp transcriptional regulators and not RXR:RAR directly. In Fig. 2B, we explore these effects on isolated wt and FM1 elements driving the expression of the heterologous TK promoter. IL-1β and JNK directly suppress wt, but not mutant, Ntcp DR2 element activity, whereas co-transfection of dnJNK abolishes IL-1β suppression of wt, but not mutant, DR2-driven TK activity. Together, these findings support the integral role of RXR:RAR on baseline Ntcp promoter activity and specify the RXR:RAR DR2 binding element as the target of the effects of IL-1β acting through a JNK-dependent signaling pathway.

Curcumin Blocks IL-1β-mediated Suppression of RXR:RAR Binding Activity and JNK Phosphorylation—Nuclear binding activity of RXR:RAR is significantly reduced in livers of rats treated with endotoxin or IL-1β-treated HepG2 cells, yet the molecular mechanisms are unknown (4, 9). HepG2 cells were pretreated with the three MAPK signal pathway inhibitors (curcumin, PD98059, or SB203580) 30 min prior to IL-1β/H9252 or saline control, and crude nuclear extracts were analyzed for RXR:RAR binding activity via electrophoretic mobility shift assay. As shown in Fig. 3A, IL-1β-mediated suppression of RXR:RAR DR2 binding activity via electrophoretic mobility shift assay. As shown in Fig. 3A, IL-1β-mediated suppression of RXR:RAR DNA binding activity was completely abrogated by pretreatment with curcumin but not with PD98059 or SB203580. Moreover, as a marker of JNK activation, these same nuclear extracts were studied for content of the Activator Protein 1 (AP-1), a known target of IL-1β and JNK signaling in...
liver and HepG2 cells (24, 25). IL-1β treatment led to robust activation of AP-1 signals, which was blocked by curcumin but not by PD98059 or SB203580. Thus, curcumin prevents the IL-1β-mediated repression of RXR:RAR binding activity as well as the activation of AP-1, consistent with interfering with JNK-dependent signaling. Total cellular RXR levels were unchanged by IL-1β or any of the three pretreatments (data not shown).

We have recently shown (19) in COS7 fibroblasts that activation of stress-activated pathways, including activation of JNK, leads to RXR phosphorylation and reduced retinoid activation of an RXR:RAR target plasmid. Whether or not these pathways apply in IL-1β-treated HepG2 cells is not known. If curcumin works via the blockade of IL-1β activation of JNK, then curcumin should inhibit the phosphorylation of JNK induced by IL-1β-dependent signaling. Because the peak of IL-1β-mediated activation of JNK, ERK, and p38 MAPK in HepG2 cells occurs at 15–30 min after treatment, lysates were made after 30 min of pretreatment with inhibitors (or Me2SO control) followed by 30 min of treatment with IL-1β (see Fig. 4A and Ref. 26). Phosphorylated JNK levels (as detected by immunoblotting with anti-phospho-JNK-specific antibodies) rise in response to IL-1β but not when HepG2 cells were treated with curcumin (Fig. 3B). Note that there was no discernable change in phospho-JNK activation by IL-1β in the presence of ERK or p38 MAPK inhibitors when compared with Me2SO control, as previously reported by Kumar et al. (26).

**IL-1β Activation of JNK Phosphorylation Correlates with Phosphorylation of RXR**—Post-translational phosphorylation of nuclear receptors has recently become recognized as a means of regulating nuclear receptor function via cross-talk with signal transduction cascades (reviewed in Ref. 27). We have recently described (19) stress-activated kinase-dependent phosphorylation of RXR in fibroblasts that leads to a significant reduction in RXR-dependent gene expression and have sought to determine whether this pathway was active in IL-1β-repressed RXR function in liver-derived HepG2 cells. Treatment of HepG2 cells with 1 ng/ml IL-1β led to a detectable increase in phospho-JNK levels at 5 min, maximal at 30 min, and a return to baseline levels by 6 h after treatment (Fig. 4A). There was no change in total JNK protein levels during the duration of the experiment. To determine whether phospho-JNK can phosphorylate RXR, we employed the technique of co-incubation of immunoprecipitated JNK with a glutathione S-transferase-linked RXR fusion protein (GST-RXR) (19). This means of detecting phosphorylated RXR does not require knowledge of phosphorylated residue(s) or currently unavailable phospho-

![Fig. 3. Effects of inhibitors on the binding activities of RXR:RAR and AP-1 and the phosphorylation of JNK. A.](Image 255x525 to 554x729)
IL-1β Suppression of the Ntcp Promoter via JNK

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Discussion

Ntcp RNA expression is markedly suppressed in response to a variety of cholestatic conditions and inflammatory signals in cell culture systems, animal models, and recently in analysis of liver biopsies from humans with liver disease (3, 4, 9, 28). In virtually every model of inflammation and cholestasis (including those with exposure to hydrophobic bile acids), Ntcp RNA levels fall rapidly and profoundly. A significant proportion of this repression appears to be due to impairments in the functioning of Ntcp transcriptional activators (8). To date, only one Ntcp transcriptional activator, the nuclear receptor heterodimer RXR:RAR, has been shown to be critical for repression by both bile acid-activated and inflammation-based regulation. The mechanism underlying the bile acid-mediated repression of RXR:RAR activation of the Ntcp promoter relies upon the bile acid-induced expression of the transcriptional repressor, SHP, which, in turn, functionally impairs RXR:RAR activation of the Ntcp promoter (10, 29–33). Of interest is the finding that IL-1β-mediated suppression of the Ntcp promoter also involves repression of RXR:RAR function, although any role for SHP in cytokine-mediated hepatic gene regulation remains controversial (9, 11, 12). In this report, we have provided evidence that the effects of IL-1β on RXR:RAR function involve direct impairment of RXR:RAR nuclear binding activity via a JNK-dependent mechanism. Moreover, we have provided evidence that curcumin is a potent JNK inhibitor that completely blocks IL-1β-mediated suppression of Ntcp promoter function and RXR:RAR nuclear binding activity, correlating with inhibition of IL-1β-induced phosphorylation of JNK and phosphorylated JNK-mediated phosphorylation of a GST-RXR substrate.

In addition to activation of JNK by stressors and cytokines, Gupta et al. (11) clearly show that hydrophobic bile acids are potent activators of JNK in primary rat hepatocytes. This is an especially relevant finding for our studies and for hepatocyte function in general, because elevated intracellular concentrations of bile acids are an obligate component of virtually all forms of chronic progressive liver disease. Moreover, it provides evidence for an additional means of regulating hepatic gene expression by bile acids. The potential role of SHP-independent regulation of Cyp7a1 and Ntcp genes is emphasized by two recent findings. First, Ntcp RNA are suppressed in endotoxin-treated mice, yet SHP RNA levels are not up-regulated. Second, bile acid feeding of SHP-null mice leads to JNK activation and suppression of Cyp7a1 RNA levels (12, 34). The intriguing results of these studies suggest that bile acids can regulate gene expression by multiple mechanisms.

We employed curcumin, a derivative of the spice turmeric, as a JNK inhibitor (13). Curcumin has been proposed to have anti-inflammatory, anti-tumor, and anti-apoptotic effects, yet a controlled examination of its efficacy in any clinical condition has yet to be published (35, 36). There are, however, numerous examples of the effects of curcumin on gene expression that support its potential role in JNK inhibition. For example, curcumin pretreatment suppresses tumor necrosis factor-α induction of AP-1-mediated activation of genes in endothelial cells (vascular cell adhesion molecule 1 and tissue factor) and fibroblasts (monocyte chemoattractant protein 1), as well as JNK activation by a variety of stress response agonists in Jurkat T cells (13, 37–39). Curcumin has effects on hepatic function relevant to our studies. Curcumin treatment of rats leads to increased bile flow, attenuation of the hyperlipidemia associated with streptozotocin-induced diabetes, and up-regulation of Cyp7a1 activity (40–43). Whether the effects of curcumin on bile flow or Cyp7a1 expression relate to JNK inhibition remains to be determined.

Curcumin can have effects on other signal transduction pathways, including inhibition of tumor necrosis factor-α activation of ERK, that may suggest targets other than JNK (13, 36). Support for invoking JNK as the primary target of curcumin’s inhibition of IL-1β signaling in HepG2 cells comes from several sources. First, curcumin inhibits JNK-mediated suppression of the wt Ntcp promoter and the isolated RXR:RAR element driving the TK promoter (Fig. 2, A and B). Second, IL-1β-mediated phosphorylation of ERK is not inhibited by curcumin in HepG2 cells (data not shown). And third, the effects of curcumin on nuclear RXR:RAR binding activity and Ntcp promoter expression are faithfully mimicked by a recently reported specific JNK inhibitor, SP600125 (data not shown) (44).

Nuclear receptor phosphorylation has been shown to be one of the more important means of post-translational regulation of receptor function (reviewed in Refs. 27 and 45). Both activation and inhibition of activity has been reported, but neither the sites nor the means of nuclear receptor phosphorylation are consistent among the different receptors, and the effects may be cell type-specific. Several groups have explored the role of phosphorylation on RXR activity, and it has proven to be complex and in some cases contradictory. Lefebvre et al. (46) have shown that inhibition of phosphatase activity by okadaic acid treatment of COS cells co-transfected with RXR and RB leads to increased basal expression of luciferase reporter plasmids driven by an isolated DR2, but not DR5 elements. These findings were correlated with increased affinity of overexpressed RXR and RB in nuclear extracts for DR2 elements derived from okadaic acid-treated COS cells. In support of phosphorylation of retinoid receptors leading to increased activity, Kopf et al. (47) found that phosphorylation can inhibit proteasome-mediated degradation of RXR:RAR heterodimers, thereby prolonging retinoid response. In contrast, we and others (19, 48) have found that activation of RXR phosphorylation by stress-activated pathways leads to reduced RXR-dependent promoter activity. Solomon et al. (48) show that human RXRα activity is reduced in transfected human keratinocytes by MAPK-dependent phosphorylation of serine 260, while we recently reported (19) that stress-mediated activation of two enzymes in the MAPK pathway, MAPK kinase 4 (MKK4) and JNK, leads to RXR phosphorylation and reduced retinoid response. In contrast, Adam-Stitah et al. (49) provide support for JNK-dependent activation of RXR-RAR activity and have mapped JNK-dependent phosphorylation of RXR to several residues in the N-terminal region and serine 265 of the mouse RXRa. Perhaps the most likely explanation for these divergent results involves disparities in experimental methods. Specifically, we rely on experiments on the role of phosphorylation of native retinoid receptors, whereas others have used co-transfected and obligately overexpressed receptors. Which RXR residues are phosphorylated by IL-1β-activated JNK is currently unknown and requires investigation.

Another recently described mechanism for down-regulation of nuclear receptor function in inflammation is AP-1-mediated competition for coactivators (e.g. CBP, cAMP-response element binding protein, CBP/p300). In addition, the JNK-induced phosphorylation of CBP and CBP/p300 may be a mechanism for the inhibition of AP-1-mediated transcription in inflammation. These examples of the effects of curcumin on bile flow or Cyp7a1 expression relate to JNK inhibition remains to be determined.

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ment-binding protein binding protein) (50). This is unlikely to be an important contributor because the expression of the FM1 mutant, which contains intact binding sites for the CBP-recruiting transactivors, hepatocyte nuclear factor 1α and CAAT/enhancer-binding protein α (C/EBPα), is unaltered by treatment with IL-1β (Fig. 2A) (9, 14, 51, 52).

In the present studies, we have linked a physiological mediator of hepatic inflammation, IL-1β, to JNK-dependent suppression of nuclear RXR-RAR binding activity, leading to down-regulation of the liver-specific Ntcp gene promoter. Although IL-1β has been linked to JNK activation in several cell types before, we believe that this is the first example of IL-1β leading to JNK regulation of any nuclear receptor-regulated gene. This may have significant ramifications in the hepatocyte, given the central role of RXR-dependent processes in a broad range of critical hepatic functions, including intermediary metabolism, digestion, and drug metabolism/detoxification/excretion (53, 54). A greater understanding of these regulatory pathways may well assist in the design and implementation of therapeutic interventions of acute and chronic liver diseases.

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