The acute phase response (APR) is associated with decreased hepatic expression of many proteins involved in lipid metabolism. The nuclear hormone receptors peroxisome proliferator-activated receptor α (PPARα) and liver X receptor (LXR) play key roles in regulation of hepatic lipid metabolism. Because heterodimerization with RXR is crucial for their action, we hypothesized that a decrease in RXR may be one mechanism to coordinate down-regulate gene expression during APR. We demonstrate that lipopolysaccharide (LPS) induces a rapid, dose-dependent decrease in RXRα, RXRβ, and RXRγ proteins in hamster liver. Maximum inhibition was observed at 4 h for RXRα (62%) and RXRβ (50%) and at 2 h for RXRγ (61%). These decreases were associated with a marked reduction in RXRα, RXRβ, and RXRγ mRNA levels. Increased RNA degradation is likely responsible for the repression of RXR, because LPS did not decrease RXRβ and RXRγ transcription and only marginally inhibited (38%) RXRα transcription. RXR repression was associated with decreased LXRα and PPARα mRNA levels and reduced RXR-RXR, RXR-PPAR and RXR-LXR binding activities in nuclear extracts. Furthermore, LPS markedly decreased both basal and 1,4,6,43-induced expression of acyl-CoA synthetase, a well-characterized PPARα target. The reduction in hepatic RXR levels alone or in association with other nuclear hormone receptors could be a mechanism for coordinately inhibiting the expression of multiple genes during the APR.

Small lipophilic compounds, such as steroids, thyroid hormones, vitamin D, and retinoids, regulate gene expression by binding to nuclear hormone receptors (1–3). Nuclear hormone receptors are the largest known family of transcription factors, with over 150 members currently. The nuclear hormone receptors share a common structural composition, including a central, highly conserved DNA-binding domain and a carboxyterminal domain that mediates ligand recognition, receptor dimerization, and ligand-dependent activation (1–3).

The nuclear receptor superfamily has been divided into four major subgroups according to their dimerization and DNA binding properties (3). Class II receptors consist of nuclear receptors that heterodimerize with the retinoid X receptor (RXR)1 and usually bind to direct repeats separated by a variable number of spacer nucleotides (3, 4). The class II subgroup includes the retinoic acid receptor (RAR), thyroid hormone receptor, vitamin D receptor, farnesoid X receptor, peroxisome proliferator-activated receptor (PPAR), and liver X receptor (LXR) (3, 5). Three distinct RXR genes have been cloned: RXRα, RXRβ, and RXRγ. RXRα is strongly expressed in liver, kidney, muscle, lung, and spleen (6, 7). RXRα is also present in the brain and heart (6, 7). RXRβ is expressed ubiquitously, and it is present at low level in liver, intestine, and testis (6, 7). RXRγ is expressed only in liver, kidney, muscle, brain, heart, and adrenal (6, 7). To date, the 9-cis retinoic acid isomer has been identified as the most potent endogenous ligand for RXR (8).

The acute phase response (APR), which is induced during infection, inflammation, and injury, is associated with numerous changes in lipid metabolism (9). Hypertriglyceridemia, decreased high density lipoprotein cholesterol levels, accelerated lipolysis, decreased hepatic fatty acid (FA) oxidation, and inhibition of the synthesis of bile acids are some of the alterations in lipid metabolism that occur during the APR (9). In most instances, these changes are mediated by pro-inflammatory cytokines such as tumor necrosis factor (TNF) or interleukin (IL)-1 and are due to alterations in gene transcription (9). However, the molecular mechanisms underlying these alterations in gene transcription that account for the changes in lipid metabolism during the APR remain to be identified.

Both PPAR and LXR have been implicated in the regulation of genes important in lipid metabolism. PPARα activation in the liver stimulates FA metabolism and transport, and LXR activation leads to an increase in bile acid synthesis. Specifically, PPARα increases the expression of carnitine palmitoyltransferase I, 3-hydroxy-3-methylglutaryl-CoA synthase, acyl-CoA oxidase, acyl-CoA synthetase (ACS), cytochrome P450 4A enzymes, FA transport protein, and FA-binding protein (10–17). Furthermore, LXR stimulates the expression of 7α-hydroxylase gene (CYP7A1), the rate-limiting enzyme for conversion of cholesterol to bile acids (18, 19). Studies in our laboratory (20–24) and others (25, 26) have shown that the expression or activity of each of these proteins involved in lipid metabolism in the liver is rapidly and markedly decreased following induction of the APR by LPS or cytokine administration.

One potential mechanism by which the expression of many

---

1 The abbreviations used are: RXR, retinoid X receptor; PPAR, peroxisome proliferator-activated receptor; LXR, liver X receptor; APR, acute phase response; FA, fatty acid; TNF, tumor necrosis factor; IL, interleukin; LPS, lipopolysaccharide; ACS, acyl-CoA synthetase; BW, body weight; IP, intraperitoneally; APP, acute phase protein; mut, mutant; HNF, hepatocyte nuclear factor.

---

* This work was supported by grants from the Research Service of the Department of Veterans Affairs and by National Institutes of Health Grants DK 49448 and AR 39639. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Veterans Affairs Medical Center, Metabolism Section (111F), 4150 Clement St., San Francisco, CA 94121. Tel.: 415-750-2005; Fax: 415-750-6927; E-mail: kfgld@itsa.ucsf.edu.
genes could be coordinately decreased during the APR is by the reduction of the levels of specific transcription factors. Because heterodimerization with RXR is crucial for the action of several nuclear hormone receptors including PPAR and LXR, we hypothesized that a decrease in RXR levels in the liver may occur during APR. Previous studies by Sugawara et al. (27) showed that TNF decreases the expression of a RXRβ promoter construct in rat GH3 cells. Here we report that RXRα, RXRβ, and RXRγ proteins and mRNA levels decline during the APR in hamster. RXR repression is associated with LXRα and PPARα repression, resulting in an overall decreased ability of RXR-RXR homodimers, RXR-PPAR, and RXR-LXR heterodimers to bind to their respective response elements.

EXPERIMENTAL PROCEDURES

Materials—LPS (Escherichia coli 55:B5) was obtained from Difco Laboratories and freshly diluted to desired concentration in pyrogen-free 0.9% saline. Human TNF-α (specific activity, 5 × 10^7 units/mg) was provided by Genentech, Inc. Recombinant human IL-1β (specific activity, 4 × 10^6 units/mg) was a gift from Dr. Charles A. Dinarello (University of Colorado, Denver, CO). The cytokines were freshly diluted to desired concentrations in pyrogen-free 0.9% saline containing 0.1% human serum albumin. Oligo(dT)-cellulose type 77F was from Amersham Pharmacia Biotech. Wy-14,643 was purchased from Sigma and freshly resuspended in corn oil at the appropriate concentration. [α-32P]dCTP (3,000 Ci/mmoll), [γ-32P]dATP (3,000 Ci/mmoll), and [α-32P]dUTP (800 Ci/mmoll) were purchased from NEN Life Science Products.

Animals—Male Syrian hamsters (140–160 g) were purchased from Simonsen Laboratories (Gilroy, CA). The animals were maintained in a normal-light-cycle room and were provided with rodent chow and water ad libitum. Anesthesia was induced with halothane. To assess the effect of the acute phase response on RXR, hamsters were injected intraperitoneally with 0.1–100 μg/100 g of body weight (BW) LPS, 25 μg/100 g of BW TNF-α, or 1 μg/100 g of BW IL-1β in 0.5 ml of saline or with saline alone. To assess the effect of LPS treatment on PPARα activation, hamsters were injected IP daily with Wy-14,643 at a dosage of 5 mg/100 g of BW or with corn oil alone for 5 days. On the fifth day, 100 μg/100 g of BW LPS or saline alone was administered IP. Food was withdrawn at the time of injection because LPS and cytokines induce marked anorexia in rodents (28). Livers were removed after treatment at the times indicated below. The doses of LPS used in this study have significant effects on triglyceride and cholesterol metabolism without causing death (23, 29). Similarly, the nonlethal doses of TNF-α and IL-1β used in this study reproduce many of the effects of LPS on lipid metabolism, causing marked changes in serum lipid and lipoprotein levels (9, 30).

Preparation of Nuclear Extracts—Fresh liver (1.5–2 g) was homogenized in 10 mM HEPES (pH 7.9), 25 mM KCl, 0.15 mM spermine, 1 mM EDTA, 2 mM sucrose, 10% glycerol, 50 mM NaF, 2 mM sodium metavanadate, 0.5 mM dithiothreitol, and 1% protease inhibitor mixture (Sigma) at the times indicated below after LPS or saline treatment. Immediately following homogenization, nuclear proteins were extracted as described by Neish et al. (31), except that 1 mM NaF, 0.1 mM metavanadate, and 1% protease inhibitor mixture (Sigma) were added to all buffers. Nuclear protein content was determined by the Bradford assay (Bio-Rad), and yields were similar in control and LPS-treated groups.

Western Blot Analysis—Denatured nuclear protein (25 μg) was loaded onto 10% polyacrylamide precast gels (Bio-Rad) and subjected to electrophoresis. After electrotransfer onto polyvinylidene difluoride membrane (Amersham Pharmacia Biotech), blots were blocked with phosphate-buffered saline containing 0.1% Tween and 5% dry milk for 1 h at room temperature and incubated for 1 h at room temperature.
with the following polyclonal rabbit antibodies (Santa Cruz Biotechnology) at a dilution of 1:5000: anti-RXRα, anti-RXRβ, and anti-RXRγ. Immune complexes were detected using horseradish peroxidase-linked donkey anti-rabbit IgG (dilution 1:20,000) according to the ECL Plus Western blotting kit (Amersham Pharmacia Biotech). Immunoreactive bands obtained by autoradiography were quantified by densitometry.

RNA Isolation and Northern Blot Analysis—Total RNA was isolated from 300–400 mg of snap-frozen whole liver tissue by a modified acid guanidinium thiocyanate-phenol-chloroform method (32) as described earlier (21). Poly(A)+ RNA was purified using oligo(dT) cellulose and quantified by measuring absorption at 260 nm. Ten micrograms of poly(A)+ or 20 μg of total RNA were denatured and electrophoresed on a 1% agarose/formaldehyde gel. The uniformity of sample applications was checked by UV visualization of the acridine orange-stained gel before electrotransfer to Nytran membrane (Schleicher & Schuell), or when indicated, p18S was used as a control probe. We and others have found that LPS increases actin mRNA levels in liver by 2–5-fold in rodents (29, 33). TNF and IL-1 produced a 2-fold increase in actin mRNA levels. LPS also produces a 2-fold increase in glyceraldehyde-3-phosphate dehydrogenase and a 2.6-fold increase in cyclophilin mRNA (20). Thus, the mRNA levels of actin, glyceraldehyde-3-phosphate dehydrogenase, and cyclophilin, which are widely used to normalize data, cannot be used to study LPS or cytokine-induced regulation of proteins in liver. However, the differing direction of the changes in mRNA levels for specific proteins after LPS or cytokine administration, the magnitude of the alterations, and the relatively small standard error of the mean make it unlikely that the changes observed were due to unequal loading of mRNA (20, 23, 24, 29, 34). Prehybridization, hybridization, and washing procedures were performed as described previously (21).

Membranes were probed with [α-32P]dCTP labeled cDNAs using the random priming technique. mRNA levels were detected by exposure of the membrane to x-ray film and quantified by densitometry. hRXRα cDNA was a gift from Dr. Daniel D. Bikle (University of California, San Francisco, CA). mouse RXRβ, mouse RXRγ, human LXRα, and human LXRβ cDNAs were kindly provided by Dr. David J. Mangelsdorf (University of Texas Southwestern Medical Center, Dallas, TX). RACS cDNA was kindly provided by Dr. Pamela J. Smith (Ross Products Division, Abbott Laboratories, Columbus, OH). rPPARα, mPPARδ, and mPPARγ cDNAs were a gift from Dr. Anthony Bass (University of California San Francisco, CA).

Nuclear Run-on Transcription Assay—Isolation of liver nuclei from fresh tissue, the procedure for in vitro nuclear transcription and hybridization was essentially as described by Clarke et al. (35). Briefly, nuclei were incubated with 200 μCi of [α-32P]UTP, and after labeling nascent transcripts for 30 min at 30 °C, total RNA was recovered according to Chomczynski and Sacchi (32). After prehybridization, all of the in vitro labeled RNA isolated (2–9 × 10^6 cpm total) from nuclei of control and LPS-treated hamsters were hybridized to prepared nylon membrane (Schleicher & Schuell). After being washed and autoradiographed, the filters were air-dried, and the amount of in vitro labeled RNA that hybridized to each dot containing 10 μg of cDNA for RXRα, RXRβ, RXRγ, actin, and vector pUC19 was measured by liquid scintillation counting.

Electrophoretic Gel Mobility Shift Assays—10 μg of crude nuclear extract were incubated on ice for 30 min with 6 × 10^8 cpm of 32P-labeled oligonucleotides in 15 μl of binding buffer (20% glycerol, 25 mM Tris-HCl (pH 7.5), 40 mM KCl, 0.5 mM MgCl2, 0.1 mM EDTA, 1 mM dithiothreitol), 2 μg of poly(dI-dC), and 1 μg of salmon sperm DNA. Double-
stranded oligonucleotide probes were end-labeled with T<sub>4</sub>-polynucleotide kinase in presence of 50 μCi of [γ-<sup>32</sup>P]dATP and purified on a Sephadex G-25 column (Amersham Pharmacia Biotech), and the LXR oligonucleotide was subsequently gel-purified. DNA-protein complexes were separated by electrophoresis (constant voltage of 300 V) on a 5% non-denaturing polyacrylamide gel in 1× TBE at 4 °C. The gel was dried, exposed to x-ray film, and quantified by densitometry. In the competition assay, a 100-fold molar excess of the specific or mutated unlabeled oligonucleotide was preincubated on ice for 1 h with 10 μg of nuclear extract from control hamster in the binding buffer before adding the oligonucleotide probe. The following oligonucleotides were used: PPAR<sup>α</sup>-response element, 5′-GATCCTCCGACCGTACCGACCTTTCTCTTT-CAGTTCCA-3′ (36); mut-PPAR<sup>α</sup>-response element, 5′-GATCCTCCGACCGTACCGACCTTTCTCTTT-CAGTTCCA-3′; CRBP<sup>II</sup>, 5′-GATACCTGCTGTCACAGTCAGTTCAA-3′ (36); (37, 38); mut-CRBP<sup>II</sup>, 5′-GATACCTGCTGTCACAGTCAGTTCAA-3′; CYP7-LXR response element, 5′-GATCCTCCGACCGTACCGACCTTTCTCTTT-CAGTTCCA-3′; CYP7-LXR response element, 5′-GATCCTCCGACCGTACCGACCTTTCTCTTT-CAGTTCCA-3′ (18); and mut-CYP7-LXR response element, 5′-GATCCTCCGACCGTACCGACCTTTCTCTTT-CAGTTCCA-3′ (18). In supershift studies, control nuclear extract was preincubated with 2 μl of one of the following antibodies (Santa Cruz Biotechnology) for 1 h at room temperature prior to the addition of the labeled probe: anti-RXRα, anti-RXRβ, anti-RXRγ, and anti-rabbit IgG.

**RESULTS**

**LPS and Cytokines Decrease RXR Levels**—We initially determined the effect of LPS administration on RXR<sup>α</sup> protein levels in the nuclei from liver of Syrian hamsters. RXR<sup>α</sup> is the most abundant RXR isoform in liver. As shown in Fig. 1A, LPS (100 μg/100 g of BW) produced a maximum decrease (62%) in RXR<sup>α</sup> protein levels at 4 h. A similar decrease was also present at 8 h following LPS treatment, but by 16 h, RXR<sup>α</sup> protein was returning toward normal levels (23% decrease at 16 h). As shown in Fig. 2A, the LPS-induced decrease in RXR<sup>α</sup> protein levels was dose-dependent, with the half-maximal effect occurring at approximately 2 μg/100 g of BW. Thus, LPS at relatively low doses (LD<sub>50</sub> for LPS in rodents is approximately 5 mg/100 g of BW) rapidly decreases RXR<sup>α</sup> protein levels in the liver of Syrian hamsters.

We next determined whether this decrease in RXR<sup>α</sup> protein levels was associated with alterations in RXR<sub>α</sub> mRNA levels in the liver. As shown in Fig. 3A, LPS administration resulted in a marked reduction (97%) in RXR<sub>α</sub> mRNA levels in the liver of Syrian hamsters at 4 h. To determine whether this decrease in mRNA levels was due to an inhibition of transcription, nuclear run-on assays were performed on nuclei prepared from hamster liver 4 h after LPS or saline injection. As shown in Fig. 4A, LPS treatment resulted in a 38% decrease in RXR<sup>α</sup> transcription compared with control. Therefore, the decrease in RXR<sub>α</sub> mRNA protein levels following LPS administration is associated with a decrease in mRNA levels that is partially accounted for by LPS inhibition of RXR<sup>α</sup> gene transcription. However, the modest reduction in transcription compared with the marked decrease in mRNA levels suggests that post-transcriptional factors in addition to inhibition of transcription contribute to the LPS-induced decrease in RXR<sub>α</sub> mRNA levels.

Because cytokines, such as TNF and IL-1, mediate many of the changes induced by LPS administration, we next examined the effects of TNF and/or IL-1 on RXR<sub>α</sub> mRNA levels. As shown in Fig. 3B, 2 h after the administration of TNF, IL-1, or TNF plus IL-1, there was a 63, 60, and 80% reduction in RXR<sub>α</sub> mRNA levels, respectively. Thus, the combination of TNF and IL-1 can reproduce the effects of LPS.

Both RXR<sub>β</sub> and RXR<sub>γ</sub> are also present in the liver but are expressed at lower levels than RXR<sub>α</sub>. As shown in Fig. 1, B and C, LPS treatment (100 μg/100 g of BW) resulted in a decrease in RXR<sub>β</sub> and RXR<sub>γ</sub> protein levels in the liver of Syrian hamsters. RXR<sub>γ</sub> was decreased by 61% as early as 2 h after LPS administration but returned to normal by 8 h (Fig. 1C). RXR<sub>β</sub> protein...
protein levels also rapidly decreased following LPS treatment, but in contrast to RXRγ, this decrease was sustained for at least 16 h (Fig. 1B). The decrease in protein levels of RXRγ and RXRβ induced by LPS was a sensitive response, with the half-maximal effect seen at less than 1 μg of LPS/100 g of BW for RXRγ (Fig. 2C) and approximately 1 μg of LPS/100 g of BW for RXRβ (Fig. 2B). Thus, LPS treatment not only decreases RXRα protein levels but also decreases the levels of RXRβ and RXRγ, which are less abundant isoforms of RXR.

To determine whether the decreases in RXRβ and RXRγ could be due to changes in mRNA levels, we next measured hepatic mRNA levels in the liver following LPS treatment. At 4 h after LPS treatment, there was a 76 and 90% reduction in the hepatic mRNA levels of RXRβ and RXRγ, respectively (Fig. 3A). In contrast to RXRα, the decreases in RXRβ and RXRγ mRNA levels were not associated with a decrease in transcription (Fig. 4B and C).

We next examined the effect of TNF and/or IL-1 on RXRβ and RXRγ mRNA levels in the liver. As shown in Fig. 3B, cytokine treatment reduced RXRβ and RXRγ mRNA levels by approximately 50%. In contrast to the effect of cytokines on RXRα mRNA levels, cytokine administration did not reduce RXRβ or RXRγ mRNA levels to the degree seen following LPS treatment.
LPS Treatment Also Reduces LXRα and PPARα Expression in Liver—To determine whether the decrease in RXR expression is associated with an alteration in LXR and PPAR expression, LXR and PPAR mRNA levels were measured in hamster liver following LPS treatment. LXRα is abundantly expressed in liver and in tissues playing an important role in lipid metabolism (39), whereas LXRβ, which is also present in the liver, displays a more widespread pattern of expression (40). PPARα has been shown to be the major isoform in human (39), rat (41), and mouse (42) liver. PPARβ and PPARγ are present in the liver but at a lower level of expression than PPARα (39, 41, 42).

Four hours after LPS administration, there was an 89% reduction in mRNA levels of LXRα and PPARα (Fig. 5, A and B, respectively). The level of expression of the minor isoforms was reduced by 84% in the case of PPARγ (Fig. 5B) and was not significantly altered in the case of PPARβ (Fig. 5A). Thus, in contrast to the overall inhibition of RXR species, LPS treatment lead to the specific inhibition of LXRα, PPARα, and PPARγ but not LXRβ or PPARδ.

LPS Administration Decreases the Binding of RXR Homodimers and RXR-PPAR and RXR-LXR Heterodimers to Specific Response Elements—Electrophoretic gel mobility assays were carried out to determine whether the decrease in RXR expression resulted in a decline in RXR binding to DNA. Nuclear hormone receptors recognize derivatives of a direct hexanucleotide repeat. The orientation of the half-sites and the number of nucleotides spacing the two half-sites determine the specificity of the response element (3, 5). We used a 32P-labeled DNA oligonucleotide containing the RXR response element, a direct repeat spaced by one nucleotide (DR1), from the retinol-binding protein type II (CRBPII) promoter, which preferentially binds to RXR homodimers (37). As shown in Fig. 6A, three major RXR complexes were observed in the control samples. Competition with a 100-fold molar excess of specific oligonucleotide, but not of mutated oligonucleotide, demonstrated the specificity of the three complexes. In addition, a portion of these complexes was supershifted after incubation of control nuclear extract with anti-RXRα and anti-RXRβ antibodies (Fig. 6C). Specifically, incubation with RXRβ antisera markedly decreased the higher molecular weight complex, suggesting that this complex is mainly composed of RXRβ homodimers. In our hands, RXRγ antisera was unable to supershift any of the three complexes. The formation of RXR-DNA complexes was not affected by nonspecific IgG. At 4 h, LPS treatment induced an overall 74% (p < 0.005) decrease in RXR homodimer binding, with the two higher molecular weight complexes being the most affected (Fig. 6, A and B). Thus, the decrease in RXR nuclear protein levels is associated with a decline in RXR binding.

**FIG. 5.** Effect of LPS treatment on LXR (A) and PPAR (B) mRNA levels in hamster liver. Syrian hamsters were injected IP with either saline or LPS (100 μg of LPS/100 g of BW). Four hours later, livers were removed, and poly(A)+ RNA and Northern blots were prepared as described under “Experimental Procedures.” Data (means ± S.E., n = 5) are expressed as a percentage of controls. ***, p < 0.005 versus control.**
binding activity during endotoxemia.

Because RXR is the obligatory partner for high affinity binding of PPAR and LXR to their response elements, we next carried out electrophoretic gel mobility assays to determine whether the down-regulation in RXR species along with PPAR and LXR binding were reduced by 90% \((p < 0.005)\) and 58% \((p < 0.05)\) compared with control, respectively. Therefore, LPS treatment leads to a global decrease in RXR, PPAR, and LXR dimer binding activity in the liver.

To determine whether the decrease in RXR-PPAR binding that we found in hepatic nuclear extracts from LPS treated animals could be associated with a decreased expression of a PPARregulated gene, we next examined the effect of LPS on ACS mRNA level in hamsters pretreated with a specific PPAR agonist, Wy-14,643 (43). As reported previously (21), LPS treatment alone resulted in a marked decrease (72%) in ACS mRNA levels in liver at 4 h (Fig. 7). Five days of treatment with Wy-14,643 markedly up-regulated (2-fold) ACS mRNA levels, as expected (11, 44). Most importantly, LPS administration led to a marked reduction in ACS mRNA levels in hamsters pretreated with Wy-14,643, indicating that LPS, possibly by decreasing RXR-PPAR heterodimer binding, can block the stimulatory effect of PPARs activators in liver.

**DISCUSSION**

Infection, inflammation, and trauma induce a wide array of metabolic changes in the liver that constitute the APR (45). The

---

**FIG. 6. Effect of LPS treatment on RXR, PPAR, and LXR binding to their specific response element.** Syrian hamsters were injected IP with either saline or LPS (100 \(\mu\)g of LPS/100 g of BW). Four hours later, hepatic nuclear extracts were prepared as described under “Experimental Procedures.” Ten micrograms of nuclear extracts were incubated with radiolabeled oligonucleotides representing binding sites for RXR homodimers, and RXR-PPAR and RXR-LXR heterodimers. A, representative electrophoretic gel mobility shift assays. Unlabeled specific (100Xsfx) and nonspecific (100Xmut) competing oligonucleotides were included at 100-fold excess 1 h prior to the addition of the labeled probes. Arrows represent specific bound complexes. B, densitometric analysis of hepatic DNA-binding proteins. Data (means \(\pm\) S.E., \(n = 5\)) are expressed as a percentage of controls. *, \(p < 0.05\); ***, \(p < 0.005\) versus control. C, electrophoretic mobility shift assay using a nuclear extract from a control hamster performed in the presence of antibodies raised against RXRa (lane 2), RXRb (lane 3), RXRg (lane 4), and rabbit IgG (lane 5). SS1 and SS2 represent the complexes supershifted by the RXRa and RXRg antibodies, respectively.
APR is mediated by cytokines, particularly TNF, IL-1, and IL-6 (46, 47). The hepatic synthesis of certain proteins, such as C-reactive protein and serum amyloid A, is increased (positive acute phase proteins), whereas the synthesis of other proteins, such as albumin and transferrin, is inhibited (negative acute phase proteins) (45).

The mechanism by which gene transcription is stimulated during the APR has been extensively studied. Class I acute phase proteins (APPs) are stimulated by IL-1 type cytokines, whereas class II APPs are stimulated by the IL-6 family of cytokines (46, 47). IL-1-induced activation of CEBP and NFκB is thought to mediate the increase in transcription of class I APP genes, whereas activation of CEBP and members of the STAT family of transcription factors is thought to mediate the IL-6-induced stimulation of class II APP gene expression (46, 47).

Much less is known about the molecular mechanisms responsible for the repression of negative APPs. Down-regulation of specific hepatic nuclear factors, such as HNF-1 and HNF-4, during APR (48) has been implicated in the regulation of certain negative APPs. For example, a decrease in HNF-1 is thought to be responsible for the reduced transcription of albumin (49), the microsomal triglyceride transfer protein (50) and the sodium-dependent bile acid transporter (51), whereas a decrease in HNF-4 could account for the decline in apoCIII levels (52, 53).

In the present paper, we demonstrate that the induction of the APR by either LPS or cytokine administration decreases the levels of all three RXR isoforms in the liver. The decrease in RXRA, RXRβ, and RXRγ protein levels occurs rapidly, within 2–4 h, and is sustained for as long as 16 h for the most abundant isoform of RXRA and also for RXRβ. Moreover, this decrease in RXR protein levels is induced by low doses of LPS (half-maximal effect occurring at approximately 1–2 μg of LPS/100 g of BW, compared with a LD₅₀ of approximately 5 mg/100 g of BW), indicating that this reduction in RXR is a very sensitive response to LPS. The decrease in RXR protein levels is accompanied by a marked reduction in RXR mRNA levels, suggesting that a decrease in protein synthesis account for the reduction in protein levels.

Interestingly, the decrease in RXR mRNA levels does not appear to be entirely due to a decrease in RXR gene transcription. Nuclear run-on assays did not demonstrate a change in RXRβ and RXRγ transcription and showed only a very modest 38% decrease in RXRα transcription, which is not sufficient to account for the marked reduction in RXRα mRNA levels following LPS administration. It therefore appears that the decrease in RXR mRNA levels is primarily due to an LPS-induced specific degradation of RXR mRNA. Recent studies have suggested that LPS also reduces connexin 32 mRNA levels in the liver by increasing their degradation rate (54). Unfortunately, using in vivo models such as these, it is difficult to carry out studies to directly demonstrate that LPS accelerates RXR mRNA degradation. In addition to the usual difficulties of measuring RNA degradation in vivo, degradation studies typically use actinomycin D, and it should be recognized that this compound dramatically increases the sensitivity to LPS (55), which will make interpretation of the results difficult. Definitive studies of the mechanism by which RXR mRNA levels are decreased during the APR await the development of an in vitro model.

The decrease in RXR protein levels in the liver during the APR may affect the transcription of a variety of genes. In the present study, we demonstrate that RXR binding to RXR-RXR response element is decreased following LPS treatment. In addition to forming homodimers, RXR is an obligate partner in heterodimers formed with several nuclear hormone receptors, such as PPAR and LXR. We further demonstrate that the...
expression of LXRα, PPARα, and PPARγ, along with the binding of nuclear extracts from acute phase liver to RXR-PPAR (DR-1) and RXR-LXR (DR-4) regulatory elements, is reduced. Because LPS treatment did not significantly modify the level of expression of LXRα and PPARγ, it would be of interest to determine whether the expression and/or the binding of other nuclear hormone receptors, such as RAR, thyroid hormone receptor, vitamin D receptor, and farnesoid X receptor, that also form heterodimers with RXR, is also reduced.

Given the variety of nuclear hormone receptors that form obligate heterodimers with RXR and the large number of genes that they regulate, a decrease in RXR and some of its partners in the liver during the APR could provide a mechanism to coordinately decrease the expression of a large number of different proteins. Looking at one model gene, ACS, which is regulated by PPARs (11), we demonstrate that LPS administration decreases ACS mRNA levels not only in normal animals but also in animals in which ACS expression was induced by prior treatment with the PPARα ligand, Wy-14,643. These data suggest that induction of the APR can inhibit the stimulation of transcription induced by PPARα activators. However, to understand the relative importance of RXR repression and LXRα or PPARα repression for the decreases in gene transcription that occur during the APR, one will have to develop transgenic models in which RXR levels are maintained during the APR.

The APR results in marked alterations in lipid metabolism in the liver (9). Whereas hepatic fatty acid uptake is increased and fatty acids are preferentially esterified to form triglycerides, there is a concomitant decrease in fatty acid oxidation and in bile acid synthesis. Many of the enzymes and transporters involved in these metabolic changes, such as carnitine palmitoyltransferase I, 3-hydroxy-3-methylglutaryl-CoA synthase, acyl-CoA oxidase, ACS, FA transport protein, FA-binding protein, and CYP7A are known to be regulated by PPAR or LXR (10–19). It is possible that during the APR, the reduced availability of RXR protein and possibly of other nuclear hormone receptors represents a mechanism to coordinately regulate these metabolic changes in liver. Additionally, it has recently been recognized that orphan receptors PXR and CAR form heterodimers with RXR and modulate drug metabolism by regulating the expression of CYP2 and CYP3 P450 enzymes (56). A decrease in RXR could by itself explain the well-characterized decrease in P450 enzymes and inhibition of drug metabolism that occurs during the APR (57). Lastly, one would anticipate that genes regulated by other nuclear hormone receptors that form heterodimers with RXR might also be downregulated during the APR. In fact, prior studies have shown that the expression of the malic enzyme, which is regulated by PPAR (58) and thyroid hormone receptor (59), is decreased after liver injury (60), supporting this hypothesis.

In summary, the present manuscript demonstrates that the APR is associated with a decrease in mRNAs coding for RXR proteins, resulting in a marked reduction in RXR protein levels in the liver. This reduction in RXR species appears to be primarily due to an increase in RNA degradation rate. RXR repression is associated with reduced LXRα and PPARα expression levels, resulting in an overall decreased binding activity to regulatory elements that recognize RXR-RXR, RXR-PPAR, and RXR-LXR dimers in nuclear extracts from acute phase liver. It can be hypothesized that the reduction in RXR levels, along with levels of other nuclear hormone receptors in the liver, could be a mechanism to coordinately down-regulate the expression of a large number of genes during the APR.

Acknowledgments—We thank Drs. David J. Mangelsdorf and Johan Auwerx for their valuable suggestions.
APR Is Associated with RXR Repression in Liver

48. Burke, P. A., Drotar, M., Luo, M., Yaffe, M., and Forse, R. A. (1994) Surgery 116, 285–292
49. Barrera-Hernandez, G., Wanke, I. E., and Wong, N. C. W. (1996) J. Biol. Chem. 271, 9969–9975
50. Navasa, M., Gordon, D. A., Hariharan, N., Jamil, H., Shigenaga, J. K., Moser, A., Fiers, W., Pullock, A., Grunfeld, C., and Feingold, K. R. (1998) J. Lipid. Res. 39, 1220–1230
51. Trauner, M., Arrese, M., Lee, H., Boyer, J. L., and Karpen, S. J. (1998) J. Clin. Invest. 101, 2092–2100
52. Fraser, J. D., Keller, D., Martinez, V., Santiso-Mere, D., Straney, R., and Briggs, M. R. (1997) J. Biol. Chem. 272, 13892–13898
53. Reddy, S., Yang, W., Taylor, D. G., Shen, X., Oxender, D., Kust, G., and Leff, T. (1999) J. Biol. Chem. 274, 33050–33056
54. Theodorakis, N. G., and De Maio, A. (1999) Am. J. Physiol. 276, R1249–R1257
55. Leist, M., Gantner, F., Bohlinger, I., Tieg, G., Germann, P. G., and Wendel, A. (1995) Am. J. Pathol. 146, 1220–1234
56. Waxman, D. J. (1999) Arch. Biochem. Biophys. 369, 11–23
57. Stanley, L. A., Adams, D. J., Lindsay, R., Meehan, R. R., Liao, W., and Wolf, C. R. (1988) Eur. J. Biochem. 174, 31–36
58. IJpenberg, A., Jeannin, E., Wahli, W., and Desvergne, B. (1997) J. Biol. Chem. 272, 20108–20117
59. Weiss, R. E., Murata, Y., Cua, K., Hayashi, Y., Seo, H., and Refetoff, S. (1998) Endocrinology 139, 4945–4952
60. Diez-Fernandez, C., Sanz, N., and Cascales, M. (1996) Biochem. Pharmacol. 51, 1159–1163