Decreased expression levels of rat liver glutathione S-transferase A2 and albumin during the acute phase response are mediated by HNF1 (hepatic nuclear factor 1) and IL6DEX-NP

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The acute phase response is characterized by positive and negative regulation of many liver proteins including GSTs (glutathione S-transferases) and albumin. The expression of albumin and some GSTs are dependent on HNF1 (hepatic nuclear factor 1). Interleukin 6 plus dexamethasone induce a nuclear protein (IL6DEX-NP) in rat hepatocytes *in vitro* that binds to a promoter element adjacent to the HNF1 site of rGSTA2 and decreases its expression. We determined how HNF1 and IL6DEX-NP regulate rGSTA2 and albumin expression in rats during the acute phase response after LPS (lipopolysaccharide) treatment. Expression of rGSTA2 and albumin mRNA decreased 3 h after LPS treatment and remained low for 48 h. Transcription rates showed a similar pattern but albumin transcription was less affected. HNF1 and IL6DEX-NP binding to the rGSTA2 promoter was present in control livers but was absent at 3 and 6 h after LPS. By 12 h, HNF1 and IL6DEX-

NP binding to the rGSTA2 promoter reappeared and increased to above normal at 48 h. The patterns of HNF1 and IL6DEX-NP binding to the albumin promoter were similar. Affinity of IL6DEX-NP for the albumin promoter was less than that for the rGSTA2 promoter and changes in the transcription rates were consistent with the difference. Early decreases in rGSTA2 and albumin during the acute phase response are due to decreased binding of HNF1. Later persistent decreases in transcriptional rate of rGSTA2 and to a lesser extent albumin are due to increased IL6DEX-NP binding. IL6DEX-NP appears to be an important negative regulator of gene expression *in vitro* and *in vivo*.

Key words: cytokine, gene regulation, sepsis, transcription, transcription factor.

INTRODUCTION

The acute phase response in animals follows a variety of adverse events including infections, burns and endotoxaemia. Changes in the levels of serum and non-serum proteins are characteristic of the acute phase response. Most of the changes in protein levels reflect changes in gene expression within the liver [1–3]. Positive acute phase proteins are those in which concentrations increase within 2–6 h of the start of the acute phase response while negative acute phase proteins are those where concentrations decrease [3]. ILs (interleukins) 1 and 6 and tumour necrosis factor- α are the principal cytokines that induce changes in liver protein expression. Type I proteins are induced by IL-1-like cytokines and include amyloid A and C-reactive protein. Type II proteins are induced by IL-6-like cytokines and include fibrinogen, haptoglobin and α_1 -antitrypsin. Glucocorticoids may also play a permissive or synergistic role affecting changes in the expression of acute phase proteins [3,4].

The induction of positive acute phase proteins are typically secondary to cytokine-mediated changes in the DNA-binding activity of transcription factors such as nuclear factor κ B, STAT (signal transducers and activators of transcription protein), activator protein 1 and CCAAT/enhancer-binding protein families. The promoter regions of positive acute phase protein genes bind activating transcription factors which cause an increase in transcriptional activity [3,5]. Negative acute phase proteins include

albumin, the sodium bile acid transporter Ntcp (sodium-dependent taurocholate-co-transporting polypeptide) [6], and some of the GSTs (glutathione S-transferases) [7,8]. The mechanisms by which negative acute phase proteins are down-regulated are not well understood but are presently being characterized. For example, decreases in the DNA-binding activity of the transcription factors HNF1 (hepatic nuclear factor 1) and RXR α :RAR α (retinoid X receptor/retinoic acid receptor) may both be involved in the decrease in expression of Ntcp [4,6,9]. HNF1 is a constitutively expressed transcription factor in hepatocytes that is necessary for the basal expression of a number of liver proteins including albumin, Ntcp and some GSTs [9–11]. Treatment of rats with LPSs (lipopolysaccharides) causes a decrease in DNA-binding activity of HNF1 by a mechanism that involves tumour necrosis factor- α and which leads to decreased expression of Ntcp and other bile-acid-transporting proteins [6,12–14]. The effect of LPS on Ntcp expression may also be mediated in part by an IL-1 β -induced decrease in the levels of the RXR:RAR heterodimer binding to the Ntcp promoter [12,15].

GSTs (EC 2.5.1.18) are a family of enzymes involved in drug metabolism and the protection of cells from injury by toxic electrophiles. There are at least seven GST classes and the Alpha class is the predominant type in the liver [16]. The GSTs and albumin are negative acute phase proteins and the expression of an Alpha class GST (rGSTA2) and of albumin decrease significantly after the administration of LPS to rats [7]. Both proteins are

Abbreviations used: DEX, dexamethasone; EMSA, electrophoretic mobility-shift assay; GST, glutathione S-transferase; HNF1, hepatic nuclear factor 1; IL, interleukin; IL6DEX-NP, IL-6-plus-DEX-induced nuclear protein; LPS, lipopolysaccharide; Ntcp, sodium-dependent taurocholate-co-transporting polypeptide; RXR α :RAR α , retinoid X receptor/retinoic acid receptor.

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dependent on HNF1 for basal expression [17,18]. Recently, IL-1 β was shown to decrease the expression of GSTs in cultured rat hepatocytes and in human Caco-2 cells [15,19]. The effect of IL-1 β in Caco-2 cells appeared to be mediated by the HNF1 site of the GST promoter [19] while in rat hepatocytes the effect of IL-1 β was post-translational on message stability [15]. IL-6 plus DEX (dexamethasone) leads to a reversible decrease in the expression of rGSTA2 in cultured rat hepatocytes. The effect of IL-6 is mediated at the level of transcription via elements in the promoter region of the rGSTA2 gene [8]. More-recent studies with transfected chloramphenicol acetyltransferase reporter constructs of rGSTA2 promoter segments in rat hepatocytes in primary culture defined a 150 bp sequence that mediates the response to IL-6 and dexamethasone. A 30 bp nucleotide from the 150 bp sequence used in EMSAs (electrophoretic mobility-shift assays) bound a nuclear protein [termed IL6DEX-NP (IL-6-plus-DEX-induced nuclear protein)] that was induced by IL-6 with DEX but was not induced in the absence of either agent. The core sequence for IL6DEX-NP binding (TGATT) is part of the HNF1 site of the rGSTA2 promoter and was hypothesized to be a negative regulator of rGSTA2 transcription [4].

Given the importance of the loss of binding activity of HNF1 in the decrease in expression of bile-acid transport proteins during the acute phase response [8–11,20], we hypothesized that the loss of HNF1-binding activity would account for some of the decrease in expression of rGSTA2 and albumin during LPS-induced acute phase response in whole animals. We also hypothesized that IL6DEX-NP would be induced in response to LPS and would also contribute to the decreased expression of rGSTA2 and perhaps albumin. To assess the effect of LPS on the expression of rGSTA2 and albumin, we measured transcriptional activity by run-on assays and steady-state mRNA levels by Northern analysis. We examined the binding activity of HNF1 and IL6DEX-NP to the negative regulatory element of rGSTA2 and to a related sequence in the albumin promoter by EMSA during the course of the acute phase response to determine whether the negative regulation was due to a loss of HNF1 binding, induction of IL6DEX-NP binding, or to both. We also determined the relative affinities of IL6DEX-NP for the rGSTA2 and albumin promoters to correlate their affinities with the observed changes in rates of transcription.

EXPERIMENTAL

Materials

LPSs (3×10^6 endotoxin units/mg, from *Salmonella typhimurium*; Sigma L 2262) were dissolved in 0.9 % saline at 37 °C (2.5 mg/ml) by direct sonication and then filter sterilized (0.22 μ m). Male rats (Sprague-Dawley; Harlan; 150–200 g) were acclimatized for 1 week and fed a defined diet (Harlan Teklad AIN-76A) with water *ad libitum*. LPS was administered intraperitoneally to experimental animals (5 mg/kg, warmed to 37 °C) and an equal volume of saline was administered to control animals. At intervals (3, 6, 12, 24 and 48 h) after LPS injection, animals were anaesthetized intraperitoneally (pentobarbital 65 mg/kg) and the liver was perfused for 5–7 min with perfusion buffer to remove blood [4]. Excised livers were snap-frozen in liquid nitrogen and stored at –135 °C. Total RNA was prepared from livers for Northern analysis [21] and nuclear proteins were extracted for EMSA by homogenizing a small piece of pulverized liver in nuclear extract buffer [4]. Details of the preparation of rat hepatocytes placed in primary culture, their treatment with IL-6 plus DEX to induce IL6DEX-NP, and the preparation of nuclear extract are fully described in [4].

Northern analysis

Northern analysis was performed as described previously [7] using total liver RNA and specific probes for rGSTA1/A2 (the cDNA probe for rGSTA2 also binds to rGSTA1 mRNA and so is referred to as rGSTA1/A2), albumin and acid glycoprotein (GTT-CTGGGTCTCTGAGCTTCC). The autoradiograms were scanned in a densitometer and signal bands were corrected for loading variability by normalization with the measured signal volumes of ribosomal protein S14 as an internal standard [17].

EMSAs

Nuclear extracts for EMSA were prepared by the methods of Staal et al. [18] and Schreiber et al. [19]. EMSAs were performed as described previously [4,22,23]. Briefly, double-stranded DNA probes (30 bp in length) were end-labelled with [γ - 32 P]ATP by T4 polynucleotide kinase. Unincorporated label was removed with a Sephadex G-50 spin column. For each reaction, 5–10 μ g of nuclear proteins were incubated for 1 h at 4 °C with 0.3–0.6 pmol of end-labelled probe, 4.5 μ g of BSA and 4 μ g of poly (dI/dC) in a total volume of 20 μ l of buffer (60 mM KCl, 12 mM Hepes, 4 mM Tris/HCl, 1 mM EDTA and 48 % glycerol, pH 7.9). In experiments involving oligonucleotide competition, unlabelled oligonucleotide (5–50-fold concentration greater than radiolabelled probe) was mixed with radiolabelled probe for 30 min before the addition of the nuclear protein extracts. Samples were run on a 4 % non-denaturing polyacrylamide gel (5 W, 4 °C) for approx. 3 h and the gel was dried under vacuum and autoradiographed.

Nuclear run-on assays

Assays were performed as described previously [24]. Briefly, nuclei were isolated at intervals from whole livers of saline and LPS-treated animals. The nuclei were isolated in a sucrose gradient, resuspended and kept until used in a storage buffer at –80 °C. After thawing on ice, 250 μ g of DNA (approx. 10^7 nuclei) were added to a transcription buffer that contained [32 P]UTP. The mixture was incubated with shaking at 26 °C for 45 min. The reaction was stopped and labelled RNA was extracted by the addition of TRI reagent (Molecular Research Center, Cincinnati, OH, U.S.A.). Cloned DNA plasmids containing partial gene sequences were linearized, denatured and blotted on to nitrocellulose filters (5 μ g/slot) that were baked at 80 °C for 2 h. Linearized and denatured plasmids (5 μ g/slot) without partial gene sequences were also blotted on the filters to control for non-specific background binding of labelled RNA to plasmid DNA sequences. Aliquots of labelled RNA [(1–2) $\times 10^7$ c.p.m.] were hybridized to the cDNA-containing filters at 60 °C for 60 h in 2 ml of hybridization buffer. The blots were washed twice for 60 min each in $2 \times$ SSC (where $1 \times$ SSC is 0.15 M NaCl/0.015 M sodium citrate) at 60 °C, treated with RNase A (10 μ g/ml) for 15 min at 37 °C, washed in $2 \times$ SSC for 15 min at 37 °C, and exposed to film.

Densitometry and statistical analysis

EMSA and nuclear run-on autoradiographs were scanned on a Bio-Rad GS-700 imaging densitometer and band volumes were quantified with Bio-Rad Quantity One imaging software (version 4.1.1). For EMSA, a same-sized sampling box was positioned over each HNF1 or IL6DEX-NP band in each lane. The nuclear extract samples were run on three different gels and the volume of the IL6DEX-NP band (or those of HNF1 for the albumin promoter element) from an IL-6-treated hepatocyte sample, which

was loaded onto each gel, was used as an internal standard to control for inter-gel exposure variations. Band volumes for run-ons were corrected for blot background, control empty plasmid background, and then normalized with the corrected volume of the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) band on the same blot. Northern blots were analysed similarly using ribosomal protein S14 as the internal standard. Values are expressed as means $\pm$ S.E.M. Statistical analysis was performed with Student's *t* test and a value of $P \leq 0.05$ was considered significant.

RESULTS

Effect of LPS on mRNA levels of acid glycoprotein, albumin and rGSTA1/A2, and on rates of transcription

We confirmed that an acute phase response occurred in the present experiments by measuring steady-state mRNA levels of acid glycoprotein, a positive acute phase protein. Acid glycoprotein levels increased to a maximum of 290-fold at 6 h after the administration of LPS and were still elevated to 60-fold at 48 h (Figure 1 and Table 1). Levels of albumin mRNA were reduced by three-quarters at 3 h, increased at 6 and 12 h and then fell again to levels that were less than one-third of the control animals. Levels of rGSTA1/A2 mRNA were reduced to less than one-third of control values throughout the 48 h period of observation. To

Figure 1 Northern blots after LPS treatment

Three animals were killed at each time point following LPS administration and total liver RNA (15 μ g) was loaded per lane and electrophoresed and transferred to nylon membranes. Radiolabelled cDNA probes for rGSTA1/A2, albumin, acid glycoprotein (AGP) and S14 were hybridized to the blot. The autoradiograms were scanned in a densitometer and signal bands were corrected for loading variability by normalization with the measured signal areas of ribosomal protein S14 as an internal standard (see Table 1).

Table 1 Time course of relative changes in steady-state RNA message levels in livers of LPS-treated rats

Each value represents the relative change with respect to 0 h; means $\pm$ S.E.M. from three animals at each time point. Values were determined by densitometry and normalized for loading using S14 values as internal standards [17]. *Significantly different from control animals (0 h), $P \leq 0.05$.

Time (h)	Relative change in steady-state RNA message level (%)		
	GSTA1/A2	Albumin	Acid glycoprotein
0	100 $\pm$ 24	100 $\pm$ 27	100 $\pm$ 25
3	37 $\pm$ 10	26 $\pm$ 1	446 $\pm$ 74*
6	31 $\pm$ 5*	72 $\pm$ 8	2916 $\pm$ 1043
12	24 $\pm$ 10*	70 $\pm$ 12	2214 $\pm$ 740*
24	15 $\pm$ 5*	12 $\pm$ 2*	3517 $\pm$ 515*
48	49 $\pm$ 24	31 $\pm$ 11	654 $\pm$ 345

Figure 2 Typical nuclear run-on slot-blot after LPS administration

Signals from control bands from plasmid DNA only (pBR322 or pBluescript; results not shown) were subtracted from the signal of the appropriate gene and normalized with the signal from GAPDH (glyceraldehyde-3-phosphate dehydrogenase).

Table 2 Relative transcription rates after LPS treatment (% of control)

Rates are relative to 0 h. Nuclei were obtained from three animals at each time point. The values shown are means $\pm$ S.E.M. *Significantly different from 0 h; **significantly different from 6 h; $P \leq 0.05$.

Time (h)	Relative transcription rate (%)	
	rGSTA1/A2	Albumin
0	100	100
6	0 $\pm$ 0*	18 $\pm$ 2*
48	18 $\pm$ 10*	72 $\pm$ 18**

determine whether changes in mRNA levels observed during the acute phase response were due to a decrease in transcriptional activity, nuclear run-on assays were performed with nuclei from control animals and from animals at 6 and 48 h after LPS administration. Transcriptional rates were reduced by 100 and 82% for rGSTA1/A2 and albumin respectively at 6 h. Transcriptional activity for albumin had begun to recover at 48 h after LPS treatment (72% of control activity) while transcriptional activity for rGSTA1/A2 remained low at 18% of the control value (Figure 2 and Table 2). Therefore, the acute phase response lasted at least 48 h and the persistent decreases in expression of rGSTA2 and of albumin were due at least in part to reduced rates of transcription.

Effect of LPS on HNF1 and IL6DEX-NP binding to rGSTA2 and albumin promoters

HNF1 is necessary for the basal transcription of albumin, rGSTA2 and Ntcp [6,9,10,20]. In addition, the nucleotide sequences of the HNF1 sites of these three genes are very similar and have putative binding sites for IL6DEX-NP (see below). Previous studies have suggested that the reduced expression of negative acute phase proteins is caused by a reduction in HNF1-binding activity [6]. IL 6 plus DEX induces IL6DEX-NP in hepatocytes, which binds to a negative regulatory sequence in the promoter of rGSTA2 leading to a decrease in the transcriptional rate of the gene [4,8]. HNF1 also binds to this same region as shown by supershift experiments but its binding activity is apparently not affected by IL-6 or DEX [4]. We were interested therefore in the relationship between the loss and/or reappearance of these two nuclear proteins and the expression of albumin and rGSTA2 mRNA. After LPS treatment, there was a rapid decrease in the binding of HNF1 to the rGSTA2 and albumin promoter sequences by 3 h. Binding of HNF1 to the rGSTA2 promoter sequence recovered to values greater than baseline within 24 h while HNF1 binding to the albumin promoter remained below baseline values up to 48 h after LPS treatment (Figures 3 and 4). However, despite the recovery of HNF1 binding by 24 h, the steady-state mRNA

Figure 3 Time course of transcription factor binding to promoter elements

Nuclear protein binding to (A) rGSTA2 promoter element and to (B) albumin promoter element after LPS administration. Lanes 1 and 2 (reading from the left) in each panel are nuclear extracts prepared from cultured hepatocytes with (+) or without (–) IL-6/DEX treatment (5 µg/lane). LPS nuclear protein extracts (5 µg/lane in A and 10 µg/lane in B) were prepared for EMSA from the livers of three individual animals.

levels of rGSTA2 remained significantly reduced (Table 1). Binding of IL6DEX-NP to the rGSTA2 promoter also decreased after the administration of LPS, returned to control levels by 12 h, and increased significantly by 48 h after LPS administration (Figures 3A and 4B). In contrast, the binding of IL6DEX-NP was less with the albumin promoter at 0 h and did not recover to baseline levels within 48 h of LPS administration (Figure 4B). Therefore, the binding of IL6DEX-NP to a negative regulatory sequence of rGSTA2 promoter was elevated at a time when mRNA levels of rGSTA2 remained reduced despite higher than normal levels of HNF1. A similar pattern was seen with the albumin promoter but the binding activities of both nuclear proteins appeared to be less than those on the rGSTA2 promoter and did not increase above baseline levels at any time point.

Binding of IL6DEX-NP to HNF1 site of albumin

We have shown previously that IL6DEX-NP binds to the HNF1 region of the albumin promoter [4] and thus could function as a negative regulator of albumin expression. The nucleotide sequences in the HNF1 sites are similar for rGSTA2, albumin and Ntcp genes; underlined nucleotides for rGSTA2 are required for the maximal binding of IL6DEX-NP [4]: rGSTA2, AGA TCA

CTA GGT AAT GAT TAA TAA; Ntcp, GAT CAA ATA AAA GAT TAA CCA GCA; albumin, TTA GTG TGG GTT AAT GAT CTA C.

We noted that the decreases in mRNA levels and rates of transcription were greater for rGSTA2 in comparison with those of albumin during the later phases of the acute phase response (Tables 1 and 2). Also, the IL6DEX-NP-binding activity using nuclear extracts from whole liver before and after LPS was less when the albumin promoter was used (Figure 4B). We questioned whether this could be due to differences in the affinity of the respective promoters for IL6DEX-NP. We used the two different promoter sequences and performed competition studies with an excess of the unlabelled albumin with labelled rGSTA2 promoter and an excess of unlabelled rGSTA2 promoter with labelled albumin promoter in EMSA (Figure 5). A 5-fold excess of the unlabelled rGSTA2 oligonucleotide prevented all of the binding of IL6DEX-NP to the albumin promoter whereas a 50-fold excess of unlabelled albumin oligonucleotide still allowed for significant binding of IL6DEX-NP to the rGSTA2 promoter. Similar results were observed for HNF1 binding to the albumin promoter, suggesting that this transcription factor also binds with greater affinity to the rGSTA2 promoter than it does to the albumin promoter (Figure 5).

Figure 4 Time course of relative changes in binding of (A) HNF1 and (B) IL6DEX-NP to rGSTA2 (●) and albumin (○) promoter elements after LPS treatment

Bands from transcription factor binding (Figure 3) were scanned in a densitometer (values are means $\pm$ S.E.M.). ¹Significantly different from 0 h value (rGSTA2); ²significantly different from 0 h value (albumin); $P \leq 0.05$.

DISCUSSION

In the current report we examined the effect of LPS administration on liver rGSTA2 expression to determine if the mechanism of rGSTA2 down-regulation *in vivo* was the same as that seen *in vitro* after combined IL-6 and DEX treatment of hepatocytes. Previous work had shown that steady-state rGSTA1/A2 mRNA levels decrease following the administration of LPS [7] but the mechanism of the decrease in message levels was not determined. We observed a decrease in transcriptional rate after LPS administration that could account for the decrease in message levels. This decrease in rate was seen as early as 6 h following LPS administration and persisted for at least 48 h. Two events appear to contribute to the decrease in transcriptional rate. In the early hours after LPS administration there is a loss in the DNA-binding activity of HNF1. HNF1 is required for the basal expression of rGSTA2 as well as that of other acute phase proteins such as Ntcp and albumin [6,9,10]. Decreases in the DNA-binding activity of HNF1 are probably due to changes in protein half-life following LPS administration [6,25]. In the current study, the DNA-binding activity of HNF1 also decreased as determined by EMSA (Figure 4A). The decrease in binding activity on the rGSTA2 promoter was maximal at 3 h but had fully recovered by 24 h. Levels of albumin mRNA also fell following LPS administration, as did the rate of transcription (Figures 1 and 2). The decrease in

Figure 5 Relative binding affinities of transcription factors for binding to the HNF1 region of rGSTA2 and albumin promoters

Unlabelled DNA from the HNF1 regions of albumin and rGSTA2 promoters were used as competition for the binding of transcription factors to the labelled rGSTA2 and albumin promoters, respectively.

transcriptional rate was also associated with a loss in HNF1-binding activity (Figure 4A). Therefore, the early decrease in the levels of many HNF1-dependent negative acute phase proteins after LPS administration may be due to decreases in the promoter-binding activity of HNF1.

However, the decrease in rGSTA2 and albumin transcriptional rates cannot be accounted for solely by the loss of HNF1 binding. The decrease in levels of rGSTA2 message persisted for 48 h (Figure 1 and Table 1) and the transcription rate for rGSTA1/A2 and to a lesser extent albumin also remained below normal for this period (Table 2). The persistent decrease in transcription rate was seen, despite full recovery of HNF1 DNA-binding activity, on the rGSTA2 promoter and to a lesser extent on the albumin promoter (Figures 3 and 4). IL6DEX-NP is a nuclear DNA-binding protein that functions as a negative regulator of rGSTA2 expression [4]. IL6DEX-NP is induced by IL-6 plus DEX in rat hepatocytes in primary culture and binds to a region of the rGSTA2 promoter that is part of the HNF1 site. We investigated if IL6DEX-NP was also induced during the acute phase response. In fact, IL6DEX-NP DNA-binding activity was present in the nuclear extracts of whole liver of control animals before the administration of LPS (Figure 3). Binding activity of IL6DEX-NP to rGSTA2 then declined for 12 h after LPS administration and recovered to above control levels at 24 and 48 h, whereas binding to the albumin promoter increased slightly by 48 h but remained below initial control levels. Therefore, at 24 and 48 h after LPS treatment, the increased binding activity of IL6DEX-NP to the rGSTA2 promoter was associated with reduced transcriptional activity and may account for the persistent reduction of rGSTA2 transcripts during the acute phase response.

We were interested in whether IL6DEX-NP also bound to the albumin promoter and therefore could function as a negative regulator of this gene. The mRNA levels and rate of transcription for the albumin gene were reduced 48 h after LPS administration, although the degree of reduction was less than that of rGSTA2. We had shown previously that IL6DEX-NP also bound to the HNF1 region of the albumin promoter [4]. In the present study

we examined the relative affinity of IL6DEX-NP for the albumin compared with the rGSTA2 promoter by performing competition experiments. The relative binding activity of IL6DEX-NP was much greater for the rGSTA2 promoter than it was for the albumin promoter (Figure 5). The greater DNA-binding affinity of IL6DEX-NP for the rGSTA2 promoter is consistent with the greater reduction in transcriptional activity of rGSTA2 compared with that of albumin (Table 2).

Several other transcription factors have been shown to mediate the decrease in expression of liver proteins during the acute phase response. The nuclear receptor RXR:RAR is necessary for the basal expression of *Ntcp*. Following the administration of LPS, the nuclear-binding activity of RXR:RAR is lost and contributes to the decrease in the expression of *Ntcp* [6,15]. The loss of RXR:RAR appears to be mediated by IL-1 β . Also, there is decreased expression of growth hormone receptor during the acute phase response that is due to an inhibition of Sp1 and Sp3 binding to the growth hormone receptor promoter [26]. Therefore, the mechanisms by which different proteins are down-regulated during the acute phase response are varied and are mediated by changes in the promoter-binding activity of a number of different transcription factors. For rGSTA2 and albumin, it appears that it is the early loss of HNF1-binding activity that leads to a rapid fall in transcriptional activity and mRNA levels early in the acute phase response. Later, as HNF1-binding activity increases there is an increase in the binding activity of IL6DEX-NP that continues to exert a negative effect on the expression of the rGSTA2 and to a lesser extent on the albumin gene. Thus there is prolonged suppression of the expression of these genes following the administration of LPS.

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