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Hepatic expression of multiple acute phase proteins and down-regulation of nuclear receptors after acute endotoxin exposure

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Abstract

Acute systemic lipopolysaccharide (endotoxin, LPS) exposure, which can lead to septic shock, enhances the hepatic expression of inflammatory and acute-phase proteins (APPs). To better understand how LPS aggravates damage, changes in hepatic gene expression after a single LPS dose was screened by using microarrays for 1176 rat genes. We detected more than 20 new potential LPS-induced APPs. Following acute LPS challenge, significant up-regulation of the steady-state mRNA levels of several important early transcription factors, such as c-jun and STAT3, and cytokine-associated genes, was observed. In contrast, RT-PCR analysis revealed marked down-regulation of the nuclear receptors RXRα, PXR, FXR, LXR, PPARα and CAR. Also genes encoding lipolytic, antioxidant as well as drug- and alcohol-metabolizing enzymes were down-regulated. These data suggest that acute LPS treatment induces important early transcription factors and co-ordinately down-regulates nuclear receptors, and that this results in altered expression of a large number of downstream genes.

Keywords: Liver gene expression; Lipopolysaccharide; Nuclear receptors; Acute-phase reaction; Hepatic injury; Microarrays

1. Introduction

Gut-derived bacterial endotoxins induce inflammation, produce an APR and may cause septic shock [1,2]. Although by now the initial events induced by LPS have been elucidated, the detailed signaling pathways and the secondary changes are partly unknown. Circulating LPS complexes with a high affinity carrier, the LBP, in itself an APP [3]. The LPS–LBP complex attaches to CD14, a glycosylphosphatidylinositol-anchored plasma membrane-associated receptor, that in turn activates TLR4 and other Toll-like receptors, which act as transmembrane transducers of LPS-induced cellular signaling [4,5]. LPS signaling through TLR4 triggers several intracellular signal transduction pathways (e.g. activation of transcription factors NF-κB and various MAPK pathways) [6], to release various cellular mediators, including the pro-inflammatory cytokines IL-1β, TNF-α, and IL-6 [7,8]. IL-1 binding to its receptor IL-1R-1 can activate the transcription factors NF-κB and C/EBP and thus increase transcription of class I APP genes. Activation of the JAK/STAT family and the C/EBP type of transcription factors is thought to stimulate class II APP gene expression via IL-6 [7,8]. In addition, negative APPs are thought to be repressed by down-regulation of certain hepatic nuclear factors HNF-1 and HNF-4 [9], and also by down-regulation of nuclear receptors RXR and PPARα in response to LPS or to IL-1β and TNF-α stimulation [10]. The genes encoding APPs have also been shown to be subject to posttranscriptional regulation [11].

Acute LPS challenge results in rapid and marked or even dramatic changes in the expression of many liver genes. Rapidly induced genes, positive APPs, include those involved in the synthesis of plasma proteins, such

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Abbreviations: APP, acute-phase proteins; APR, acute-phase response; LPS, lipopolysaccharide; LBP, LPS-binding protein; TLR, Toll-like receptor; I-κBα, inhibitory protein-κBα; CDK4, cyclin-dependent kinase 4; GST, glutathione S-transferase; LACS, long chain acyl-CoA synthetase; SCYA, small inducible cytokine A3 precursor; GPX, glutathione peroxidase; PLA2, phospholipase A2; RXR, retinoic X receptor; CAR, constitutive androstane receptor; PXR, pregnane X receptor; PPAR, peroxisome proliferator-activated receptor; FXR, farnesoid X receptor; LXR, liver X receptor.

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as α1-acid glycoprotein and C-reactive protein. Typical negative APPs, such as albumin, antithrombin III and apolipoproteins, are at the same time down-regulated between 20 and 80% [12,13]. In addition, many other genes besides those encoding transcription factors and nuclear receptors have been found to be associated with LPS-induced APR. For example, genes encoding lipid metabolizing enzymes, antioxidant genes and P450 enzymes are generally down-regulated [3,12,14]. Both LBP [3] and CD14 [15] belong to the more than 30 APPs identified in liver. The response gets weaker after repeated acute LPS challenge, suggesting development of tolerance [16]. This is reflected in the effect of LPS on experimental liver injury. An acute dose of LPS aggravates injury [17,18]. However, we previously observed that chronically elevated LPS levels, which were associated with distinct effects on the expression of both pro- and anti-inflammatory cytokines, did not aggravate alcohol-induced liver changes [19]. The present study, which focused on the acute LPS effects, was undertaken to better understand how LPS affects hepatic gene expression in general.

Earlier studies on the response to LPS stimulation have analyzed only a few genes by using Northern blotting, RT-PCR, or ribonuclease protection assays. More recently, the cDNA microarray technique based on rat and canine models [20,21] has been applied. In the present study, we focused on the effect of acute LPS by using stress-associated arrays. Pertinent changes were verified by RT-PCR. Our results indicate that acute LPS treatment induces important early transcription factors and coordinately down-regulates nuclear receptors, and that this results in altered expression of a large number of down stream genes.

2. Materials and methods

2.1. Animal treatment

Adult male Wistar rats were treated with LPS as described elsewhere [19]. A bolus dose of LPS (0.5 mg/kg, *Salmonella abortus equi*, Sigma Chemical Co.) was injected intraperitoneally 4 hr before termination. The dose and response time was chosen based on literature data as to yield an acute LPS effect that has resulted at this time point in major changes in mRNA expression, but not yet in significant damage [22]. Controls were treated with saline. At termination liver samples were taken, immediately frozen in liquid nitrogen and stored at −70°. The studies were approved by the Committee of Animal Experimentation of the National Public Health Institute, Finland.

2.2. RNA isolation and Northern blot analysis

About 80 μg of total RNA was isolated from 30 to 40 mg of liver tissue by using the Atlas™ Pure Total RNA Labelling System (CLONTECH). Total RNA was electrophoresed on 1.25% agarose gel containing 20 mM guanidine thiocyanate as described [23]. Separated RNAs were blotted onto nylon membranes [24] and their quality assessed from the intactness of ribosomal RNA bands. The membranes were further hybridized with ^{32}P-labeled mouse target cDNAs generated by PCR using the primers listed in Table 1. The intensity of the target bands was normalized by the 18S ribosomal RNA band that served as an internal standard.

2.3. cDNA expression microarrays

About 80–100 μg of total RNA pooled from three liver samples in the same group were treated with DNase I enzyme. Fifty micrograms of DNA-free total RNA from each group was converted into ^{32}P-labeled cDNA probes using the Atlas™ Pure Total RNA Labeling System (CLONTECH). Each set of cDNA probes, containing the same amount of radioactivity (1.2 or 3.5×10^6 cpm/5 mL) was separately hybridized to the Atlas™ Rat 1.2 or Stress Arrays (CLONTECH). After overnight hybridization, the arrays were washed, exposed for 1, 3 or 14 days and analyzed by the Fuji Bas-1800 phosphoimager (Fujifilm). The AIDA Array Metrix software (Raytest Isotopenmessgeraete GmbH) was used for quantification of the signals. The labeling intensity of each gene was subtracted from the background and then normalized by using 22 housekeeping and non-altered genes for the Data from Rat

Table 1
Sequences of the different primers and the sizes of PCR products

mRNA	Sense primers (5′–3′)	Antisense primers (5′–3′)	Target (bp)	Mimic (bp)
RXRα	TTTCCTGCCGCTCGACTT	GGTCTTTGCGTACTGTCC	500	298
PXR	CCATGTTGGCCTTGTACA	TCACTGTGAAACACCGCA	509	316
CAR	ATACTGTCAGCAGAAGCC	GTACTGGAACCCTACATG	467	216
PPAR	GCAATGCACTGAACATCG	TTTCAACGCCGTTGGCTA	509	216
LXR	GGATAGGGTTGGAGTCAT	GTCAACAATCTCCTGCAC	646	316
FXR	GACAAAGAAGCCGCGAAT	GTGGTCCAGTGTCTGAAA	618	316
STAT3	GAACATGGAGGAGTCCAA	TGTCTAGCCAGACCCAGA	517	298
Cyclophilin	TGAGCACTGGGGAGAAAG	AGGGAATGAGGAAAATA	521	210
GSTM2	CCTTGATCAACACCGTAT	TCCTCCGAAAATCAGTGA	407	–
*CYP*2E1	CCTGGATCCAGCTTTACAATAA	AACAGGTCGGCCAAAGTCAC	253	–
*CYP*1A2	TCTTCTGGAGCATTTTGCTA	CACAAAGGGGTCTTTCCACT	300	–

1.2 Array. Four housekeeping genes were used to normalize the data obtained from Rat Stress Array. A firm limit of detection could not be established due to the variable extent of hybridization effectiveness for the various gene probes. We found empirically that signal of AU 1.0 detected by the scanner used can clearly be identified visually and this was used as the detection limit. We considered that a change in amount of signal detected by the scanner of 2.3-fold with a significant signal seen under the control situation in case of induction and under treatment conditions in case of suppression, allowed statistical treatment of the data as to determine whether significant changes in expression level had occurred.

2.4. cDNA synthesis and competitive PCR

0.9 μg of each intact total RNA sample was reverse-transcribed to cDNA by using the Advantage™ RT-for-PCR™ kit (CLONTECH). PCR mimics were constructed according to the manufacturers instruction and the competitive PCR was carried out as described before [19,25]. The sequences of the primers used and the sizes of PCR produces are listed in Table 1. The optimal conditions for the competitive PCR are given in Table 2. PCR products run on agarose gel were scanned with the Fuji Las-1000 luminescent image analyzer (Fujifilm). The intensity data of the scanned bands were converted to ratios of target to mimic, then normalized by the ratio of cyclophilin that served as a control for the quantity of RNA and efficiency of reverse transcription.

2.5. Statistical analysis

Every sample was examined at least two times by PCR or Northern blotting. The results represent means ± SD, and significance of differences was analyzed by the Student's *t*-test. *P* values <0.05 were considered as significant.

3. Results

We analyzed changes in gene expression patterns obtained 4 hr after acute LPS injection by using Atlas™ Rat 1.2 and Stress Arrays. Detectable expression was obtained for about 30% of the genes (350 out of 1176) on the Rat 1.2 Array (Fig. 1). No signals from the negative DNA controls were seen after extended (14 days) array exposure, indicating absence of genomic DNA contamination. Genes with a more than 2.3-fold difference in expression (either above 230% or below 43% of controls) after LPS treatment as well as some LPS-related and housekeeping genes are listed in Table 3 (Rat 1.2 Array) and Table 4 (Rat Stress Array). Altered expression was observed for many genes encoding proteins important for signal transduction, drug-, alcohol- and lipid-metabolism, as well as of enzymes with antioxidant properties. Particularly marked induction was observed with respect to a limited number of genes, in particular those encoding proteins of importance for cellular signaling. These included c-jun, STAT3, I-κBα, interleukin-1 receptor type I (IL-1R-1), Max (i.e. c-*myc* dimerization partner and coactivator), protein tyrosine phosphatase PTPase and interferon inducible protein 10 (IP-10). In contrast, the signaling proteins RXR and a calmodulin-dependent protein kinase were down-regulated to ∼50% of control levels. Marked induction after

Table 2
Optimal competitive-PCR conditions

mRNA	Annealing temperature (°)	$MgCl_2$ (mM)	PCR cycles
RXRα	60	1.5	37
PXR	59	1.5	34
CAR	57	1.8	35
PPAR	58	1.5	34
LXR	60	1.5	34
FXR	59	1.8	35
STAT3	60	1.5	35
Cyclophilin	53	1.7	34

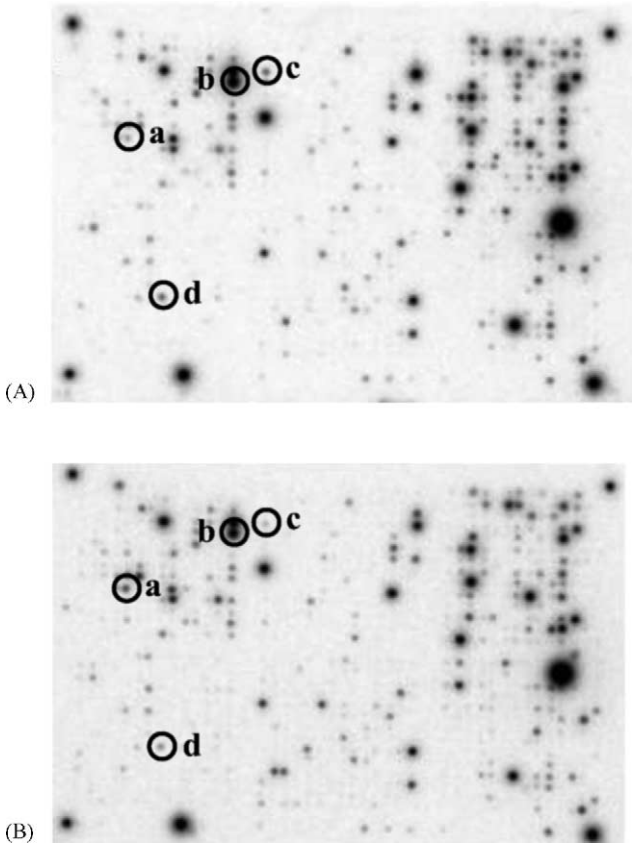

Fig. 1. Autoradiograph image of Atlas™ Rat 1.2 Arrays hybridized with ^{32}P-labeled cDNA probes prepared from enriched total RNA isolated from the liver of control (A) rats, and rats treated with acute (B) LPS. Circles and corresponding alphabets in the figure are indicating some key genes (a: signal transducer/activator of transcription 3 (STAT3), b: glutathione S-transferase Yb subunit (GSTM2), c: cytochrome P450 2E1, d: retinoid X receptor alpha). A complete list of the name and location of 1176 genes on the array is available from http://atlas.clontech.com.

Table 3
The mRNA level of hepatic-expressed genes in rats after acute LPS administration

Gene/protein name	Control	Acute LPS	
	AU	AU	%
Transcription factors and DNA-binding proteins			
Max c-*myc* dimerization partner and coactivator[a]	4.4	14	309
I-κB (I-kappa B) alpha chain	24	62	258
Signal transducer/activator of transcription 3 (STAT3)	20	75	376
Id-3 DNA-binding protein inhibitor[a]	45	142	316
Cell cycle regulators			
Prohibitin (PHB)[b]	24	0	
Immune system proteins			
Proteasome delta subunit precursor subunit Y	42	34	81
Proteasome component C13 precursor[a]	0	11	
Oncogenes and tumor suppressors			
c-jun proto-oncogene transcription factor AP-1	0	12	
Stress response proteins			
Glutathione S-transferase Yb subunit (GSTM2)	1368	506	37
Glutathione S-transferase P subunit (GST7-7)	25	0	
Glutathione S-transferase subunit 13	39	28	73
Glutathione transferase, subunit 8	48	38	79
NADPH-cytochrome P450 reductase (CPR)	18	12	69
*CYP*2E1	54	9.7	18
*CYP*1A2	23	5.3	23
Ion channel and transport proteins			
Fibroblast ADP/ATP carrier protein[b]	54	21	38
Gamma-aminobutyric acid (GABA) transporter 2[b]	11	4	36
Urate transporter/channel[a]	0	13	
ATP synthase, subunit c, P2 gene	61	32	53
Trafficking and targeting proteins			
Epidermal fatty acid-binding protein (E-FABP)[a]	40	111	278
Fatty acid-binding protein (liver L-FABP)	631	252	40
Nonspecific lipid-transfer protein precursor	182	62	34
Fatty acid binding protein, brain	21	4.6	22
Metabolic pathways			
Long chain acyl-CoA synthetase 2 (LACS2)	73	19	26
Medium chain acyl-CoA dehydrogenase precursor[b]	21	0	
Alcohol dehydrogenase class 1 (ADH1)[b]	11	0	
Cytochrome *c* oxidase, subunit Va, mitochondrial	63	46	73
3-Ketoacyl-CoA thiolase A and B[b]	34	0	
*CYP*17[b]	9.6	0	
*CYP*2C11	90	104	115
*CYP*3A1	52	11	21
*CYP*2C7	72	21	29
*CYP*4A3	46	12	25
*CYP*2C22	19	16	86
*CYP*4F1	47	24	51
*CYP*4F4	45	33	74
*CYP*4A1	6.6	4.8	73
*CYP*2C23	174	64	37
*CYP*2A1[b]	29	11	37
Cytosolic thymidine kinase (TK1)[a]	11	28	253
Adenylate kinase 3[b]	22	7.5	34
Liver arginase 1 (ARG1)[b]	30	12	40
Mitochonodrial carnitine *O*-palmitoyltransferase Ii[b]	32	0	
Long chain-specific acyl-CoA dehydrogenase[b]	32	0	
Very long chain acyl-CoA dehydrogenase precursor[b]	19	0	
NADP+ alcohol dehydrogenase	220	141	64
Receptors			
Interleukin-1 receptor type I (IL-1R-1)	0	2.4	
Growth hormone receptor precursor (GH receptor)	1.8	3.6	200
Retinoid X receptor alpha (RXR alpha RXRA)	50	22	43
Small inducible cytokine A3 precursor (SCYA3)[a]	0	74	
Insulin-like growth factor binding protein 1 (IGFBP1)	0	37	
Gastric inhibitory polypeptide precursor (GIP)	33	76	229
Stimulator, effectors and intracellular transducers			
Calcium/calmodulin-dependent protein kinase type I[b]	13	3.9	30
Cyclin-dependent kinase 4 (CDK4)	14	8.8	63
Protein tyrosine phosphatase PTPase[a]	0	28	
Calcium-dependent phospholipase A2 (PLA2)[b]	80	34	42
Interferon inducible protein 10 (IP-10)	0	155	
Protein turnover			
Thrombin[b]	1140	410	36
Aminopeptidase B[b]	10	0	
ATPase, proteasomal, liver, TBP1[b]	80	31	39
Plasma proteinase inhibitor alpha-1-inhibitor III[b]	54	18	34
Housekeeping genes			
Polyubiquitin	1430	1516	106
Phospholipase A2 precursor PLA2G1B	22	17	78
HPRT	12	5.9	49
Glyceraldehyde 3-phosphate dehydrogenase	2.7	5.1	189
Cytoplasmic beta-actin (ACTB)	44	57	130
40S ribosomal protein S29 (RPS29)	1792	1326	74

Note. AU = image intensity from Atlas Rat 1.2 Array (Arbitrary Units); % = percent change compared to control (100%). 0 = non-detectable.
[a] Putative positive APPs.
[b] Putative negative APPs.

acute endotoxin was also observed for several genes encoding non-signaling proteins, namely Id-3 (DNA-binding protein inhibitor), epidermal fatty acid-binding protein, cytosolic thymidine kinase (TK1) and urate transporter.

Table 4
The mRNA level of antioxidant enzymes in rat liver after acute LPS treatment

Gene/protein name	Control	Acute LPS	
	AU	AU	%
Cu–Zn SOD1	12.1	4.0	33
Liver catalase[a]	15.8	0.6	3.8
Microsomal glutathione S-transferase (MGST1)	12.4	11	87
Glutathione S-transferase Yb subunit (GSTM2)	25.3	10	40
Glutathione peroxidase precursor (GPX1)[a]	5.4	1.1	20

Note. AU = image intensity from Atlas Rat Stress Array (Arbitrary Units); % = percent change compared to control (100%).
[a] Putative negative APPs.

Many genes encoding enzymes involved in redox processes and in the metabolism of e.g. alcohol, pharmaceuticals and lipids were found to be down regulated. Rat Stress Array hybridization data revealed that also genes encoding antioxidant proteins, such as GSTM2, Cu–Zn superoxide dismutase (SOD)1, catalase and glutathione peroxidase precursor (GPX1) were down-regulated after acute LPS challenge (Table 4). The genes encoding alcohol dehydrogenase class 1 (ADH1) and the ethanol-inducible *CYP*2E1 were both down-regulated, as were the mRNA levels of the cytochrome P450s (*CYP*s) 1A2, 2A1, 2C7, 2C23 and 3A1. Transcripts of a number of enzymes involved in lipid metabolism were also reduced. These included CYP4A3, calcium-dependent phospholipase A2 precursor (PLA2), long chain acyl-CoA synthetase 2 (LACS2), mitochondrial carnitine *O*-palmitoyltransferase II precursor, acyl-CoA dehydrogenase precursors and 3-ketoacyl-CoA thiolases.

Application of the cDNA array technique suggests that the acute phase response to LPS in the liver involves activation of 8 genes and suppression of 21 genes, which we identify as genes (marked in Tables 3 and 4) encoding positive or negative APPs. Since gene expression on the mRNA level does not always reflect the relative expression on the protein level, the final answer to the question whether these gene products can be classified as APPs has to await analysis of their expression at the protein level. However, the gene products tentatively identified as positive APPs were proteasome component C13 precursor, urate transporter, SCYA3, protein tyrosine phosphatase PTPase, Max, Id-3, epidermal fatty acid-binding protein, and TK1. Furthermore, 21 putative negative APPs were detected. These include ADH1, the antioxidant enzymes GPX1 and catalase, as well as the lipid metabolizing enzymes PLA2, mitochondrial carnitine *O*-palmitoyltransferase II, medium, long, and very long chain acyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolases A and B. Also CYP2A1, CYP17, prohibitin, aminopeptidase B, fibroblast ADP/ATP carrier protein, GABA transporter 2, adenylate kinase 3, ARG1, plasma proteinase inhibitor alpha-1-inhibitor III, calcium/calmodulin-dependent protein kinase type I, thrombin, and liver proteasomal ATPase appear to be down-regulated by acute LPS.

To confirm the results obtained by the microarray technique, the relative mRNA level of selected target genes was determined by traditional methods (Table 5). GSTM2 mRNA was decreased 57% by acute LPS as analyzed by Northern blotting (Fig. 2), in agreement with data obtained by microarray analysis from the same samples. Changes in mRNA levels of *CYP*2E1 and *CYP*1A2 also corresponded to the microarray data (Table 5). Results based on the more sensitive competitive PCR technique also confirmed microarray data: STAT3 expression increased 4.2-fold and RXRα decreased to 46% after acute LPS challenge. These data suggest that our results based on cDNA microarray analysis can be considered as relatively reliable.

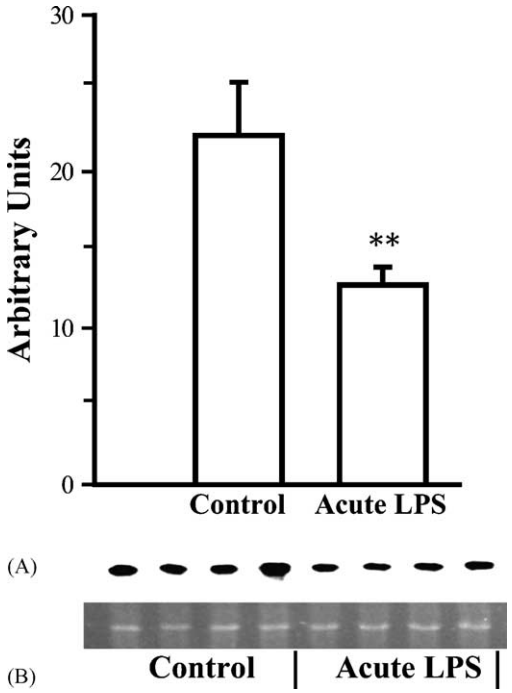

Fig. 2. Analysis of mRNA expression of GSTM2 by Northern blotting in the liver of control rats, and rats treated with acute LPS. Each group was consisted of 4 animals. The bars represent mean ± SD. $^{**}P < 0.01$ vs. control group. (A) a 1.1 kb specific band of GSTM2 was detected after 6 hr exposure with a phosphorimaging screen. (B) 18S ribosomal RNA band from denaturing agarose gel stained with ethidium bromide (0.5 μg/mL).

Our observation, based on the microarray technique, that acute endotoxin treatment causes down-regulation of the RXR expression, prompted us to investigate the effects on other nuclear receptors. Using competitive RT-PCR, we quantified the expression of the RXR partners CAR, PXR,

Table 5
Comparison of the relative mRNA levels of selected genes obtained from microarrays and competitive PCR or Northern blot analysis

mRNA	Rat 1.2 Array (%)	Rat Stress Array (%)	Northern blotting (%)	Competitive PCR (%)
GSTM2	37	40	57**	–
*CYP*2E1	18	–	20*	–
*CYP*1A2	24	–	27*	–
STAT3	376	–	–	425**
RXRα	43	–	–	46**

Note. % = percent change compared to control (100%); –: not detected.
* $P < 0.05$ vs. control group.
** $P < 0.01$ vs. control group.

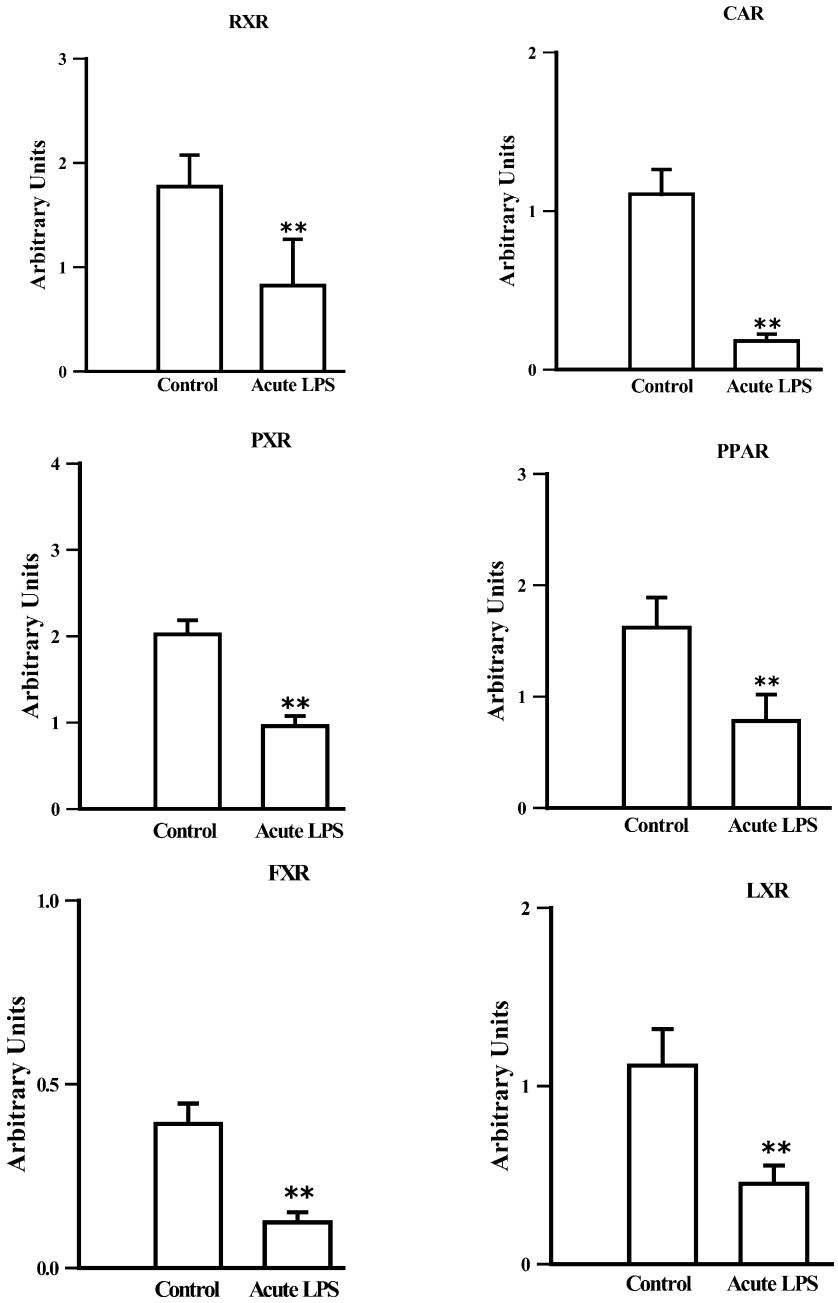

Fig. 3. Relative mRNA expression of RXRα, CAR, PXR, PPARα, FXR and LXR in the liver of control rats, and rats treated with acute LPS. The data based on competitive RT-PCR analysis as descried in Section 2. Mean ± SD (N = 5) are given. $^{*}P < 0.05$ $^{**}P < 0.01$ vs. control group.

PPAR, FXR and LXR. Acute endotoxin treatment was found to significantly ($P < 0.01$) down-regulate all these nuclear receptors in an apparently coordinated fashion (Fig. 3).

4. Discussion

By application of the cDNA microarray technique we have been able to detect 53 genes with distinctly changed (≥2.3-fold) steady-state mRNA levels after acute LPS challenge. Among these, 8 positive and 21 negative genes were identified as putative new APP genes responding to LPS treatment in rat liver. Similar data have been reported for four of these (urate transporter, alcohol dehydrogenase class 1, long-chain specific acyl-CoA dehydrogenase and liver catalase) [20]. but to our knowledge, the 25 others have not been previously identified. Whereas several of the eight new positive APPs may have roles in cell signaling, the majority of the new negative APPs are enzymes and several of them are involved in lipolysis. Because more than 80% of the liver mass consists of hepatocytes, it is anticipated that the majority of the observed changes in gene expression reflects differences in the hepatocyte mRNAs. The functional importance of the changes observed have to be addressed in further studies.

LPS elicits changes in cellular signaling at at least two different levels, namely the primary response of the cells to LPS itself and the secondary response to secondary mediators like the cytokines IL-1β, IL-6 and TNF-α. The secondary response is temporally distinct from the primary response, although it still may occur within one hour from LPS challenge. In the primary response, two key factors are NF-κB and c-jun. I-κBα, the NF-κB inhibiting protein, binds to NF-κB domains important for the nuclear translocation. Increased I-κBα mRNA in response to acute LPS in our model may reflect rapid re-synthesis and might explain the transient period of activation of NF-κB during the APR [26]. Likewise, c-jun mRNA was detected after acute LPS challenge. This is likely the result of JNK-dependent LPS-induced phosphorylation of c-jun, constitutively present at low levels, and/or ATF-2, constitutively expressed at comparatively high levels. The c-jun promoter contains a variant AP-1 element that binds c-jun/ATF-2 heterodimers or ATF-2 homodimers to transactivate c-jun [5].

Another transcription factor that was found to be affected in the present studies is STAT3. Interestingly, STAT3 was originally isolated as an IL-6 sensitive transcription factor, and IL-6 is still one of the strongest inducers of STAT3 activation, i.e. homodimerization and nuclear translocation of the transcription factor. Several APP genes but also the Stat3 gene itself have been described to be transcriptionally activated by STAT3 [26]. It is therefore likely that the increase in STAT3 mRNA showed by microarrays and competitive PCR in the present studies is part of a secondary response of the liver to LPS, mediated by cytokines such as IL-6. It has also been reported that STAT3 homodimers may complex with c-jun to regulate, e.g. expression of α_2-macroglobulin [27], which is a well-known APP. It is, therefore, a distinct possibility that STAT3/c-jun complexes are of importance during the acute LPS response of the liver. In addition, we found that acute LPS treatment can turn on or increase the expression of IL-1R-1 receptor as well as cytokine-related IGFBP-1, IP-10, which are in line with previous reports [28,29].

The nuclear receptors RXRα and PPARα have been implicated in the down-regulation of certain negative APPs. It was recently reported that in hamster liver either LPS or cytokines produced as a consequence of LPS (IL-1β and TNF-α) could induce a rapid dose-dependent decrease in both protein and mRNA levels of RXRα, RXRβ and RXRγ. The RXR repression was associated with decreased LXRα and PPARα mRNA levels, but not with LXRβ and PPARβ mRNAs. Furthermore, LPS also reduced the expression of acyl-CoA synthetase, a well characterized PPARα target and lipid metabolizing enzyme [30]. Since heterodimerization with RXR is essential for the action of several nuclear hormone receptors, including PPAR, LXR, PXR, and CAR, the decrease in hepatic RXR alone or in association with other nuclear hormone receptors during the APR, has been suggested as a mechanism to coordinately regulate the expression of a large number of different proteins [10]. Interestingly, we also noticed that acute LPS in our model decreased the mRNA level of RXRα, as well as PPARα. Moreover, not only acyl-CoA synthetase, but also other PPARα-regulated proteins such as CYP4A3 as well as liver and brain fatty acid-binding proteins were down-regulated by LPS. Furthermore, several other key enzymes in lipolysis were also found to be affected in the present study. These include the mitochondrial carnitine *O*-palmitoyltransferase II, affecting substrate availability for β-oxidation of fatty acids, and also acyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolases A and B, important enzymes of the β-oxidative pathway. Interestingly, the down-regulation of expression of at least four enzymes involved in fatty acid hydrolysis in the liver, a process previously thought to be regulated mainly by substrate and cofactor availability, may in fact be controlled by nuclear receptors, especially LXR [31] and CAR [32], both of which were down-regulated by acute LPS in the present study. This, together with the down-regulation of RXRα and PPARα, may explain how APR induced by LPS is associated with various changes in lipid metabolism [33].

Additionally, our cDNA microarray indicated LPS-mediated down-regulation of the expression of several drug-metabolizing enzymes, including CYP1A2 and CYP2E1 (also confirmed by Northern blotting), as well as CYP2C7, CYP2C23, and CYP3A1. This is consistent with previous reports, suggesting that IL-1, TNF-α and IL-6 participate in this down-regulation [14,34,35]. However, this effect could also be mediated via nuclear receptors, which control the levels of the cytochromes P450. In fact, the decrease in RXR, PPARα and PXR in our model could by itself explain the observed down-regulation of CYP2C7, CYP2C23 and CYP3A1, as well as inhibition of drug metabolism occurring during the APR [36]. Earlier studies have also indicated that nuclear factor HNF-1α is involved in regulation of CYP1A2 [37] and CYP2E1 [38], whereas the regulation of CYP2C and CYP3A subfamily members has been associated with nuclear receptors PPARα [39] and PXR [40], which are heterodimerized with RXR. Whether HNF-1α is decreased in our model is still an open question.

Oxidative stress can be induced by inflammatory cytokines after LPS treatment [41]. We observed that LPS reduced the mRNA expression of not only GSTM2, which has been identified as a negative APP in rat liver [12], but also of several other antioxidant genes, including Cu–Zn SOD1, GPX1 and catalase. This seemingly coordinated effect will severely reduce the cellular defence against oxidative stress, and contribute to LPS-induced cellular injury. Among the affected enzymes the marked down-regulation of the GPX precursor may be of particular importance [42], in addition to that of GSTM2, which apparently does not involve NF-κB [43]. A more detailed analysis of the nuclear factors/receptors involved in the LPS-induced down-regulation of antioxidant genes seems warranted.

Table 6
Effect of acute LPS exposure on the mRNA level of some important genes

Proteins/genes in different fields	Acute LPS
Signal transduction (c-jun, STAT3)	↑
RXRα, CAR, FXR, SXR, PXR, PPARα	↓
Lipolysis enzymes	↓
*CYP*s (1A2, 2C7, 2C23, 3A1)	↓
Antioxidant enzymes	↓
Alcohol metabolizing enzymes (ADH1, *CYP*2E1)	↓
GH receptor and GIP	→ or ↑

We have summarized our main findings in Table 6. While there is marked primary up-regulation of many transcription factors and cytokine-related proteins, there is primary down-regulation of nuclear receptors involved in lipid, sterol, bile acid and drug metabolism. Most probably as a consequence of this primary regulation there secondary down-regulation of a number of drug, alcohol, lipid metabolizing enzymes as well as antioxidant enzymes. We also identified eight mRNAs that respond to acute LPS as putative positive APPs, and 21 mRNAs as putative negative APPs, including several lipolytic enzymes of the β-oxidative pathway.

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