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# DOWN-REGULATION OF MULTIPLE CYTOCHROME P450 GENE PRODUCTS BY INFLAMMATORY MEDIATORS *IN VIVO*

## INDEPENDENCE FROM THE HYPOTHALAMO-PITUITARY AXIS

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**Abstract**—Inflammatory stimuli suppress constitutive hepatic expression of the *CYP2C11* and *CYP2C12* genes in male and female rat livers, respectively. Because growth hormone (GH) is an important regulator of P450 gene expression in the rat, the effects of bacterial endotoxin injection in hypophysectomized rats were compared to those in normal animals. In intact females, 0.2 mg/kg endotoxin suppressed total P450 and hepatic expression of CYP2C6, CYP2C7, CYP2C12, and CYP2E1 mRNAs, as well as CYP2C12 and CYP2E1 proteins, measured 16 hr later. CYP2C7 and CYP2E1 mRNAs were most affected (17 and 13% of untreated levels, respectively). Endotoxin treatment also induced the mRNA for the hepatic acute phase protein, haptoglobin, to 260% of control female levels. In hypophysectomized females supplemented with GH infusion, endotoxin caused the same or greater effects on expression of the P450 and haptoglobin gene products than were observed in the intact animals. It is concluded that the P450 suppression observed after endotoxin administration can occur independently of an effect on pituitary hormone secretion.

Activation of the immune system results in a decreased ability of humans and experimental animals to metabolize drugs *in vivo* [1]. In animals, infection by viruses [1–3], bacteria [4, 5] or parasites [6] are accompanied by decreases in drug-metabolizing activities of the hepatic endoplasmic reticulum, and by reduced levels of cytochrome P450, the family of hemoproteins that serve as the terminal oxidases for the microsomal drug-metabolizing system [7]. Similar effects are seen in animals treated with antigens or adjuvants [8, 9], bacterial lipopolysaccharide (LPS)† [10, 11], or agents that cause localized inflammatory reactions [11, 12].

In rats injected with bacterial LPS, levels of the constitutively expressed CYP2C11 and CYP2C12 mRNAs and proteins are suppressed [13], and at least for CYP2C11 this is achieved primarily at the level of gene transcription [14]. Local inflammation caused by turpentine has comparable effects [13, 14]. However, LPS potentiates the induction of certain forms of inducible P450 in mouse liver [15]. *In vivo* studies have implicated interferons [16–18], and interleukin-1 (IL-1) [19] in the down-regulation of P450 gene products during infection and inflammation. IL-1 and IL-6 have also been shown to suppress expression of various P450s in hepatocytes [20, 21], and hepatoma cells [22].

Treatment of animals with LPS also results in profound changes in pituitary hormone secretion [23]. Of particular relevance to *in vivo* effects on P450 gene expression, is that growth hormone (GH) secretion is inhibited initially and then stimulated [23]. IL-1 [24] and IL-6 [25] stimulate release of GH and other hormones from cultured pituitary cells, whereas the dominant *in vivo* effect of IL-1 upon the hypothalamic control of GH release may be inhibitory via stimulation of somatostatin release [26]. GH is the primary physiological regulator of many P450 genes in rodent liver [27, 28]. Therefore, many of the effects of LPS, and of cytokines released upon LPS stimulation of monocyte/macrophages, on P450 gene expression *in vivo* could be due to an alteration in normal GH regulation of P450.

In the present study, I tested whether down-regulation of P450 gene expression by LPS and inflammatory cytokines is related to modulation of plasma GH, by comparing the effects of LPS in intact rats and hypophysectomized (Hx) rats supplemented with GH. Similar effects were observed in both groups of animals, consistent with the hypothesis that P450 suppression by LPS is independent of the pituitary gland.

### MATERIALS AND METHODS

*Animals and treatments.* All procedures were approved by the Institutional Animal Care and Use Committee. Female Sprague–Dawley rats were obtained from Charles River Laboratories (Raleigh, NC) at 35 days of age. The date of delivery was designated day 0. Hypophysectomy was performed by the vendor, and its success was confirmed by ensuring that animals did not gain weight in the first 6 days of the experiment. The Hx rats were given

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† Abbreviations: LPS, lipopolysaccharide; Hx, hypophysectomized; HpG, haptoglobin; IL-1, interleukin-1; IL-6, interleukin-6; and GH, growth hormone.

0.5% dextrose and 0.9% NaCl to drink throughout the experiment. On day 6, and each day thereafter, the Hx rats received subcutaneous injections of L-thyroxine sodium, 10 μg/kg, and hydrocortisone acetate, 400 μg/kg, at 8:00 a.m. Both compounds were obtained from the Sigma Chemical Co., St. Louis, MO. On day 9, the Hx rats were implanted with subcutaneous osmotic minipumps (Alzet, Palo Alto, CA) filled with recombinant human GH (2 IU/mg, Genotropin, donated by Kabi Peptide Hormones AB, Stockholm, Sweden) to deliver 60 μg/day continuously. At 5:00 p.m. on day 15, Hx and age-matched intact rats received an intraperitoneal injection of 0.2 mg/kg LPS (*Escherichia coli* serotype 0127:B8, chromatographically pure, Sigma) or saline vehicle, and all rats were killed 16 hr later.

*Preparations and assays.* Pyrophosphate-washed hepatic microsomes were prepared by differential centrifugation [29]. Total RNA was prepared by the method of Chomczynski and Sacchi [30].

Total microsomal protein was determined as described by Lowry *et al.* [31]. Total microsomal P450 content was determined from the CO difference spectrum of the reduced protein [32]. Relative levels of CYP2C12 and CYP2E1 apoproteins in microsomal preparations were measured by Western blot assay as described previously [13]. Antibodies to rabbit CYP2E1 were donated by Dr. D. R. Koop of Oregon Health Sciences University. Band intensities were measured by laser densitometry, and the values for individual rats were calculated per gram of liver. Relative levels of P450 proteins were then expressed as a percentage of the mean for the control group.

The relative levels of CYP2C12 mRNA in total hepatic RNA were quantified by slot blot assay using the cloned cDNA C-6 as described [13]. For CYP2E1 slot blots, the full-length cDNA [33], donated by Dr. F. J. Gonzalez of the National Institutes of Health, was used. Relative levels of the mRNAs for CYP2C6, CYP2C7, and haptoglobin (HpG) were measured using $^{32}$P-5′-end-labeled oligonucleotides complementary to the following residues of the respective mRNAs: CYP2C6, nucleotides 628–667 [34]; CYP2C7, nucleotides 1501–1526 in the 3′-untranslated region [34]; HpG, nucleotides 210–239 [35]. The sequences used in the oligonucleotide probes were used to scan the Genbank and EMBL databases for homologous sequences, and no homology of more than 55% was detected to any known rodent sequence. The hybridization and high-stringency washing conditions for the oligonucleotide probes were calculated as described in Ref. 36. The intensities of the autoradiographic bands were quantified by laser densitometry. Results are expressed as the ratio of a specific mRNA to total poly (A)$^+$ (measured using an oligodeoxythymidylate 30-mer probe [19, 37]) in the samples.

*Statistical analyses.* Data from Western blot and slot blot assays are expressed as the percentage of the mean of the control group. One-way analysis of variance and the Dunn Multiple Comparison Test were used to determine differences among treatment groups. Differences were considered to be significant if $P < 0.05$. Data are presented in the figures as means ± SEM.

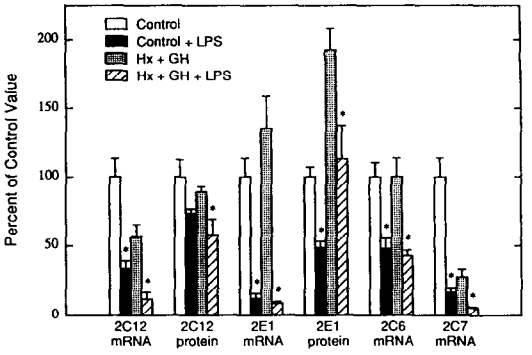


Fig. 1. Suppression of multiple P450 gene products by LPS treatment in livers of normal and Hx female rats. Groups of 4–6 adult female rats were injected with LPS or saline, and killed 16 hr later for measurement of relative levels of hepatic P450 gene products as described in the text. Age-matched Hx rats received hGH infusion from osmotic minipumps, and hydrocortisone and thyroxine injections, for 6, 9 and 9 days, respectively, before LPS injection as described in Materials and Methods. Values represent the means ± SEM for each group. Key: (*) significantly different from the appropriate saline-treated group, $P < 0.05$.

## RESULTS

Administration of endotoxin to female rats causes a decrease in hepatic CYP2C12 mRNA and protein levels that reaches a maximum at about 12 hr and persists for 48 hr [13]. To examine the effects of endotoxin on other P450 gene products in female rat liver, and to assess the role of the pituitary gland in the observed effects, LPS was administered to intact female rats, and to Hx female rats that received supplementation with hydrocortisone, thyroxine and continuous GH infusion. Rats were killed 16 hr after the LPS injection, for analysis of their liver P450s.

As observed previously [13], LPS caused significant decreases in hepatic CYP2C12 mRNA (to 34% of control, Fig. 1), as well as in total microsomal P450 (to 70% of control, Fig. 2) in intact female rats. The mRNA for CYP2C6 was decreased to a similar extent as the CYP2C12 mRNA, whereas the CYP2C7 and CYP2E1 mRNAs showed even greater decreases (to 17 and 13% of control values, respectively (Fig. 1)]. CYP2C12 and CYP2E1 apoprotein levels were less affected than were their cognate mRNAs (Fig. 1). In contrast, levels of the mRNA of the hepatic acute phase protein, HpG, were increased by LPS treatment to 260% of control female levels (Fig. 2). LPS treatment caused significant decreases in liver weights in both intact and Hx rats (to 92 and 74% of saline-treated controls, respectively), and caused an 18% increase in microsomal protein yield in the Hx animals only (Fig. 2). No effect was observed on the poly (A)$^+$ content of total hepatic RNA (Fig. 2). Both intact and Hx animals injected with LPS had decreased body weights, compared to saline-injected rats, at the time they were killed (92 and 94% of controls, respectively; data not shown).

In Hx female rats given continuous GH replace-

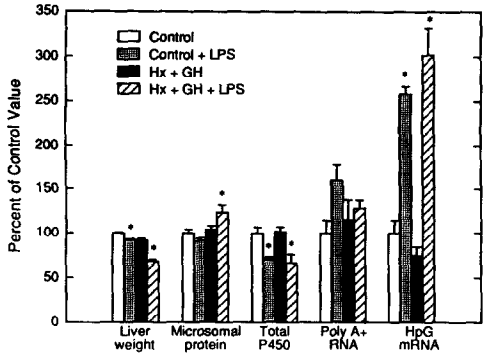


Fig. 2. Effect of LPS injection on various hepatic parameters in intact and Hx rats. Rats were treated as described in the legend of Fig. 1. Liver weights were calculated as a percentage of total body weight. Total P450 was calculated per gram of liver. Microsomal protein yield, and poly $(A)^+$ RNA and HpG mRNA contents were measured as described in the text. All results are expressed as a percentage of the control group mean. The absolute values for liver weight, microsomal protein yield and microsomal P450 content of the liver in the control group were 5.7% body weight, 5.3 mg/g liver and 4.61 nmol/g liver, respectively. Values represent the means ± SEM for each group. Key: (*) significantly different from the appropriate saline-treated group, $P < 0.05$.

ment, the effects of LPS on all P450 gene products studied were the same or greater than the effects in intact females (Figs. 1 and 2). Likewise, HpG mRNA was induced by LPS in Hx rats to a similar or greater extent compared to intact animals. These results indicated that alteration in pituitary GH secretion is not necessary for suppression of hepatic P450 gene expression by LPS.

Hypophysectomy of female rats without GH replacement causes a suppression of expression of CYP2C12 [38] and CYP2C7 [39], and elevates hepatic levels of CYP2E1 [28]. CYP2C6 is unaffected [39]. Infusion of GH to Hx animals, mimicking the female pattern of GH secretion, typically reverses the effects of hypophysectomy [28, 38, 39], although not always to control female levels [38]. In the present study, GH treatment of the Hx rats did not fully restore hepatic levels of CYP2C12 and CYP2C7 mRNAs, nor CYP2E1 protein, to normal female levels (Fig. 1), although levels of CYP2C12 protein were not different from untreated female values.

## DISCUSSION

The major conclusion from the work presented here is that modulation of serum GH is not a primary mechanism for the acute phase suppression of P450 gene expression following a systemic inflammatory stimulus. Despite the fact that cytokines have been shown to down-regulate P450 gene products in hepatocyte cultures with a constant hormonal environment [20, 21], it was important to consider the potential contribution of hormonal effects to the *in vivo* suppression of P450 gene products by inflammatory stimuli. Serum GH levels, and the temporal pattern of serum GH variation, modulate the expression of many or most constitutively expressed P450s in both positive and negative directions [27, 28]. Furthermore, endotoxin causes a biphasic effect on serum GH [23], presumably because interleukins 1 and 6 stimulate release of hypothalamic and pituitary hormones [24–26]. Because indistinguishable effects of endotoxin on total P450 and multiple P450 gene products were obtained in Hx female animals with an experimentally controlled hormonal environment and in intact rats, we can conclude that GH modulation is not necessary for this effect to occur in intact rats.

Importantly, it was also observed that induction of HpG mRNA by LPS treatment was unaffected by hypophysectomy and GH replacement therapy. This finding is not surprising since GH is not known to be important in regulation of HpG, but it demonstrates that the Hx + GH treated animals were able to mount a normal hepatic acute phase response to the inflammatory stimulus.

Also novel to this study was the finding that endotoxin treatment suppressed multiple P450 mRNAs in female rat liver. Down-regulation of CYP2C12 mRNA and of apoprotein were somewhat smaller in magnitude than observed in my previous study [13], perhaps due to the use of a lower dose of LPS (although the dose used here previously gave a maximal response in male rats [13]). CYP2C6 mRNA was suppressed to a similar extent by LPS, but CYP2C7 and CYP2E1 mRNAs were affected more profoundly. In this study, both CYP2C12 and CYP2E1 proteins were less affected by LPS treatment than their mRNAs, a phenomenon already reported for CYP2C11 in males but not for CYP2C12 in females [13]. The discrepancy for CYP2C12 may be due to the lower dose of LPS used in the present work.

Although we did not include a group of untreated Hx rats in this study, the effects of hypophysectomy alone on expression of P450 gene products are well characterized [28, 38, 39]. In the present study, some of the GH-sensitive P450 gene products were restored to normal female values by the GH treatment (CYP2C12 protein and CYP2E1 mRNA), while others were only partially restored (CYP2C7, CYP2C12 mRNAs and CYP2E1 protein). The lack of a complete feminization of P450 gene expression by continuous GH infusion has been reported before [38]. This does not affect the major conclusion of the present work, since the Hx + GH group was used as the control for statistical analysis of the effect of LPS in the Hx + GH + LPS group, and the results therefore do not depend upon a fully feminized phenotype in the GH-treated animals.

From the data presented herein, the possibility cannot be excluded that central actions of inflammatory cytokines contribute in some way to the down-regulation of P450 by LPS in intact rats. However, the present data indicating that P450 suppression can occur in the absence of changes in GH secretion, together with the documented actions of IL-1 and IL-6 on P450 expression in hepatocyte cultures [20, 21], support the hypothesis that *in vivo* suppression of P450 gene expression during

inflammation is a result of the direct actions of inflammatory mediators on the liver.

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