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Suppression of Constitutive Cytochrome P-450 Gene Expression in Livers of Rats Undergoing an Acute Phase Response to Endotoxin

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SUMMARY

Administration of purified bacterial lipopolysaccharide (LPS) to male rats suppressed the constitutive hepatic expression of the male-specific cytochrome P-450 [AH, reduced flavoprotein:oxygen oxidoreductase (RH hydroxylating), E.C.1.14.14.1] isozyme P-450h (P450IIC11) to about 35% of control levels within 24 hr. The mRNA for P-450h was more rapidly and more profoundly suppressed than was the protein, indicating (a) that the decrease in the mRNA was responsible for the suppression of the protein and (b) that other mechanisms work to maintain expression of P-450h apoprotein in the face of repression of its mRNA. Suppression of P-450h expression was maximal at an endotoxin dose of 30–100 μg/kg, indicating that P-450 suppression is concomitant with the acute-phase response of hepatic secretory proteins. The female-specific cytochrome P-450 isozyme, P-450i (P450IIC12), was suppressed to 17% of control levels by LPS administration in female rats. Suppression of the P-450i apoprotein by LPS, and recovery of its expression, was more rapid than was suppression of P-450h in males. P-450i protein and mRNA levels were concomitantly suppressed by LPS, indicating that although there is a pretranslational component to the suppression, other mechanisms may also contribute. Calculations based on estimations of the microsomal contents of P-450h and P-450i relative to the total cytochrome P-450 in untreated rat livers indicate that suppression of these forms contributes significantly to the decreases in total microsomal P-450 after LPS treatment. In these studies, hepatic microsomal NADPH-cytochrome c reductase ($TPNH_2$-cytochrome *c* reductase, E.C.1.6.2.4) activities and content of cytochrome b_5 were decreased by LPS administration in both male and female rats. Like its effects on cytochrome P-450 expression, endotoxin suppression of NADPH-cytochrome *c* reductase activities and cytochrome b_5 levels was more rapid in female rats than in males. The production of a local inflammatory response in male rats by subcutaneous injection of turpentine caused effects on cytochrome P-450, P-450h expression, and cytochrome b_5 that were similar to those of endotoxin but were less rapidly achieved.

The products of the P-450 gene superfamily (1) are the central catalysts of the hepatic microsomal drug-metabolizing system. The remarkable versatility of this system is due not only to the large number of isozymes expressed in the livers of any one species [approximately 25 have been identified in rat livers (1)] but also to the low substrate specificities of most of these forms (2, 3) and the differential induction of subsets of them by drugs (2). Some isozymes are only expressed significantly after chemical induction, whereas others are expressed constitutively (2, 3). The constitutive enzymes thus provide a host defense against foreign compounds in the absence of chemical induction. It is becoming increasingly apparent that the constitutive expression of many P-450 isozymes in the rat is hormonally regulated, with somatotropin playing a major role in that regard (4–7).

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People with viral, as well as parasitic and bacterial, infections exhibit a decreased capacity to metabolize drugs, as evidenced by decreased rates of elimination (8). In rodents subjected to bacterial infections (8, 9) or inflammatory stimuli (10–14), an impaired ability to metabolize drugs *in vivo* is accompanied by a depletion of total hepatic P-450 content and a decreased microsomal metabolism of drug substrates. These effects can be mimicked by administration of a single dose of endotoxin, the bacterial (LPS) molecule that initiates the febrile and acute phase responses typical of bacterial infection (15–17).

The acute phase response to inflammation or infection in humans and animals is characterized by an increased hepatic synthesis and secretion of a variety of secretory proteins, the "acute phase proteins" (18–21), and a decreased synthesis and secretion of a battery of "negative acute phase proteins" (18–20, 22). These acute phase responses are at least partially due to effects on gene transcription (20). The humoral mediators of the acute phase response are cytokines released from acti-

ABBREVIATIONS: P-450, cytochrome P-450; P-450h, P450IIC11; P-450i, P450IIC12; IL-1 and IL-6, interleukins 1 and 6; LPS, bacterial lipopolysaccharide.

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vated mononuclear leukocytes (19, 23). Both IL-1 and IL-6 are released from macrophages in response to endotoxin or inflammation (24) and can stimulate synthesis of acute phase proteins in isolated hepatocytes or hepatocytes in primary culture (25, 26). IL-1 has been implicated in depression of hepatic P-450 levels by LPS. C3H/HeJ mice, which do not secrete IL-1 in response to LPS, showed no effect of LPS on their hepatic drug metabolism but did respond to IL-1 injected *in vivo* with decreases in P-450 levels and drug-metabolizing activities (17, 27). Moreover, IL-1 inhibited P-450-dependent 7-ethoxycoumarin *O*-deethylase activity in isolated murine hepatocytes (17).

Although the effects of inflammation and infection on total hepatic microsomal P-450 content and drug-metabolizing activities are well documented, there is a paucity of knowledge of the effects of these pathophysiological states on expression of individual cytochrome P-450 genes and the mechanisms involved. Recently, Stanley *et al.* (28) described the effects of LPS on constitutive and inducible hepatic P-450 gene expression in mice. They found that constitutive P-450 expression was repressed by an LPS dose of 1 mg/kg but not by lower doses that have been demonstrated to produce a maximal acute phase response. Conversely, LPS at the lower doses potentiated the induction of P-450 isozymes by phenobarbital and 3-methylcholanthrene. It is difficult to interpret the results of the latter study with respect to effects on known isozymes, however, because the authors used cDNA and antibody probes developed to rat P-450s, and the mouse isozymes and mRNAs detected by these probes have not been identified.

In this paper, I have examined the effects of a single injection of *Escherichia coli* LPS on the expression of mRNAs and proteins from the rat P-450h and P-450i genes. P-450h and P-450i are constitutively expressed in a sex-specific manner in adult male and female rats, respectively (3). The most important physiological factor known to direct normal sex-specific expression of both of these isozymes is the temporal pattern of secretion of somatotropin from the pituitary gland (3–5). Here, I demonstrate that a single dose of endotoxin represses the expression of constitutive cytochrome P-450s by both pre- and posttranslational actions and that near-maximal effects on P-450h expression are attained at doses of LPS in the range required to elicit a maximal acute phase response.

Materials and Methods

Animals and treatments. Sprague-Dawley rats, 8–9 weeks old, from Harlan Sprague-Dawley Inc. were used in all experiments. They were allowed free access to food and water at all times. Chromatographically pure *E. coli* LPS, serotype 0127:B8 (Sigma), was dissolved by sonication in sterile 0.9% NaCl and injected intraperitoneally in a volume of 1.0 ml/kg of body weight. Where indicated, turpentine (NASCO Solvent Corp.), 5 ml/kg, was injected at a single subcutaneous site on the back of each rat. All control animals received sterile saline by the appropriate route. Animals were sacrificed by CO_2 asphyxiation.

Preparation of hepatic microsomes and total RNA. Livers were excised and perfused with cold 1.15% KCl. Pyrophosphate-washed microsomes were prepared as described previously (4) and were stored at −80°. Total RNA was prepared from portions of the same livers according to the method of Chomczynski and Sacchi (20) and was stored at −80°.

Assays of microsomal proteins. Total microsomal protein was determined by the method of Lowry *et al.* (30). Cytochrome b_5 content was assayed from the difference spectrum of NADH-reduced and oxidized microsomes at 424 nm (31), using an extinction coefficient of 112 mM^{-1}. Total microsomal P-450 concentrations were determined from the CO difference spectrum of the reduced protein (31). NADPH-cytochrome *c* reductase activities were assayed at 25° by a modification of the method of Phillips and Langdon (32).

Western blot immunoassays. Relative levels of P-450 isozymes h and i in microsomal samples were measured by a modification of a previously described method (4). Microsomal proteins were separated by polyacrylamide gel electrophoresis (7.5% polyacrylamide) in the presence of sodium lauryl sulfate and blotted electrophoretically on nitrocellulose filters. The filters were blocked with buffer containing 1% bovine serum albumin and probed with specific immunoabsorbed polyclonal antibodies to P-450h (4) or monoclonal antibody to P-450i (33). The antigen-antibody complexes were visualized using an appropriate horseradish peroxidase-labeled second antibody (Jackson Immunoresearch Laboratories, Inc.) with 4-chloro-1-naphthol as substrate. The intensities of the stained bands were measured using an LKB Ultroscan laser densitometer. Care was taken to ensure that signals generated were within the range giving a response proportional to the amount of microsomal protein applied to the gel.

RNA slot-blot assays. Relative levels of P-450h and P-450i mRNAs in total hepatic RNA samples were quantitated using cloned cDNAs 1.1 (34) and C-6 (35), respectively. Total RNA was denatured with formaldehyde, and three different amounts of each sample were applied to a Nylon 66 filter in the wells of a slot-blot apparatus. After the RNA was immobilized by baking or UV irradiation (UV-Stratalinker; Stratagene), the filters were probed with the ^{32}P-labeled cDNA (Multiprime; Amersham) fragments and washed under conditions shown to produce specific detection of P-450h and i mRNAs, respectively (34, 35). The filters were then subjected to autoradiography, and the intensities of the signals were quantified by densitometry. Again, care was taken to ensure that the signal was proportional to the amount of RNA applied to the filter. Relative levels of α2u-globulin mRNA were quantitated in a similar manner using a cloned cDNA probe to a murine orthologue, which was kindly donated by Dr. Gunnar Norstedt of the Karolinska Institute. All results were normalized to the relative content of β-actin mRNA in the samples, to correct for differences in total mRNA content of the samples and for differential binding of the samples to the filters.

Statistical analysis. One-way analysis of variance and Dunnett's test were used to test for significant differences between the mean of each treatment group and the mean of the control group. All results are expressed as the mean ± standard error for each group of animals. All values reflect means of individual determinations from the number of rat livers specified.

Results

Time course of LPS effects in male rat liver. A single intraperitoneal injection of 1 mg/kg LPS caused an observable decrease in total microsomal cytochrome P-450 content within 12 hr (Table 1). The effect reached a maximum at 24 hr, when microsomal P-450 levels were 69 and 79% of control values (two experiments), and was not significantly reversed for at least 72 hr after the injection. The magnitude of this effect on total P-450 content was somewhat smaller than those reported from other studies in rats (15, 16). However, in the present study the hepatic microsomal protein content, as measured by the recovered yields, was concomitantly decreased by the LPS treatment (Table 1). Consequently, the total hepatic content of P-450 was decreased by 44–61% 24 hr after LPS in the two experiments (data not shown).

The microsomal content of cytochrome b_5 was also reduced by the LPS treatment, reaching its lowest levels (79% of control) at 24 hr after injection (Table 1). This decrease was even greater (45%) when cytochrome b_5 content was expressed per gram of liver (data not shown). Similarly, NADPH-cytochrome *c* reductase activities, a measure of NADPH-cytochrome P-450 reductase content, of the microsomes were re-

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TABLE 1

Temporal dependence of the effect of LPS on hepatic microsomal protein yield, NADPH-cytochrome c reductase activities, and contents of cytochrome b_5 and P-450 in male rats

Rats were given a single intraperitoneal injection of 1 mg/kg LPS and were sacrificed at the times indicated. Total microsomal contents of cytochromes b_5 and P-450 are expressed as nmol/mg of microsomal protein. NADPH-cytochrome *c* reductase activity is given as nmol cytochrome *c* reduced/mg of microsomal protein/min. *n* is the number of rats in each group.

Time after injection	*n*	Microsomal protein	Total P-450	Cytochrome b_5	NADPH-cytochrome c reductase
hr		*mg/g of liver*	*nmol/mg*	*nmol/mg*	*nmol/mg/min*
Expt. 1					
0	4	6.7 ± 0.2	1.01 ± 0.03	0.70 ± 0.05	103 ± 3
6	4	6.2 ± 0.1	0.94 ± 0.03	0.74 ± 0.03	120 ± 8
12	4	4.7 ± 0.6[a]	0.79 ± 0.03[a]	0.61 ± 0.05	96 ± 6
24	4	3.7 ± 0.3[a]	0.70 ± 0.02[a]	0.57 ± 0.03	87 ± 4
Expt. 2					
0	4	5.6 ± 0.2	1.07 ± 0.02	0.94 ± 0.03	139 ± 7
24	4	4.0 ± 0.3[a]	0.85 ± 0.03[a]	0.72 ± 0.02[a]	114 ± 8
36	3	3.6 ± 0.1[a]	0.80 ± 0.02[a]	0.74 ± 0.01[a]	108 ± 6[b]
48	3	3.7 ± 0.1[a]	0.84 ± 0.04[a]	0.79 ± 0.03[b]	100 ± 5[a]
72	4	3.7 ± 0.3[a]	0.88 ± 0.05[a]	0.78 ± 0.05[b]	94 ± 7[a]

[a] Significantly different from control group, $p < 0.01$.
[b] Significantly different from control group, $p < 0.05$.

duced by the LPS treatment (Table 1). A significant decrease (22%) was first observed after 36 hr, but the mean values also tended to be reduced at earlier time points. NADPH-cytochrome *c* reductase activities were still depressed at 72 hr (68% of control levels). When expressed per gram of liver, the activity of this enzyme was decreased by 38% within 12 hr and was still reduced by 56% after 72 hr (data not shown).

Microsomal levels of the male-specific P-450h isozyme were also suppressed by endotoxin administration, in a time-dependent manner. A significant decrease in microsomal P-450h levels was observed 12 hr after administration (Figs. 1 and 2). The effect was near maximal at 24 hr, but levels continued to decline slightly up to 72 hr after administration, when they reached 52% of control values (Fig. 2). When expressed per gram of liver, P-450h levels followed the same temporal pattern, although the magnitude of the effect was greater (maximum decrease to 35% of control male levels; Fig. 2). LPS treatment suppressed microsomal P-450h levels to a slightly greater extent than it decreased total P-450 levels. This effect was clearest at

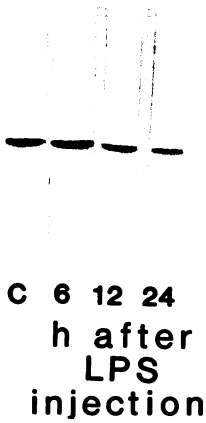

Fig. 1. Western blot showing repression of P-450h expression in male rat liver by endotoxin. Four micrograms of hepatic microsomal protein from control rats (C) or rats killed at the indicated time after a 1 mg/kg injection of LPS were subjected to polyacrylamide gel electrophoresis in the presence of sodium lauryl sulfate and blotted on a nitrocellulose filter. The filters were probed with antibodies to P-450h as described in the text. The figure is a composite of nonadjacent lanes taken from a single blotting experiment.

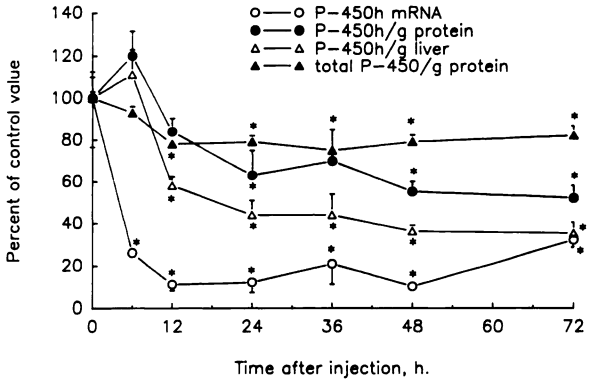

Fig. 2. Time course of repression of P-450h expression in male rat livers by endotoxin. Rats were given a single intraperitoneal injection of 1 mg/kg LPS and sacrificed at the times indicated. Relative hepatic levels of total P-450, P-450h apoprotein, and P-450h mRNA were determined as described in the text. For P-450h apoprotein, relative levels were calculated per g of microsomal protein and per g of liver. Relative levels of P-450h mRNA in total hepatic RNA were normalized to the hepatic content of β-actin, as described in the text. Total P-450 levels were calculated per g of microsomal protein. All values are given as percentages of the mean values for the control male group. The number of animals in each group is given in Table 1. This graph summarizes both experiments shown in Table 1. * Significantly different from control, $p < 0.05$.

72 hr after treatment, when total P-450 levels were 82% of control compared with 52% for P-450h (Fig. 2).

Levels of P-450h mRNA were suppressed by endotoxin administration (Fig. 3). This effect was rapid, with a decrease of 74% occurring only 6 hr after LPS injection, and was maximal (89% decrease) after 12 hr (Fig. 2). P-450h mRNA levels did not begin to return toward control levels until 72 hr, when they were still reduced by 68%. The fact that suppression of P-450 mRNA levels preceded that of P-450h protein expression and that the magnitude of the effect on the mRNA was greater than that on the protein at steady state indicates that the suppression of P-450h mRNA is primarily due to an effect on P-450h gene transcription or mRNA stability. However, the observation that P-450h protein levels are less affected than its mRNA suggests that LPS may act by other mechanisms to increase expression of P-450h protein relative to its mRNA.

P-450i is a female-specific isozyme that is only expressed at very low levels in male rats (3, 5). Levels of P-450i mRNA in

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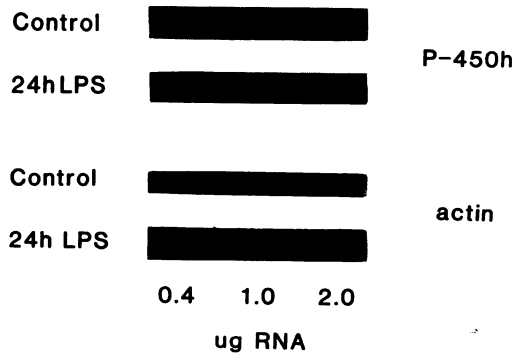

Fig. 3. RNA slot blot showing repression of P-450h mRNA by LPS in male rat liver. Total hepatic RNA from control rats and rats injected with 1 mg/kg LPS 24 hr previously was applied to Nylon 66 filters in the amounts indicated. The blots were probed with labeled cDNAs for P-450h and β-actin, as described in the text, and subjected to autoradiography.

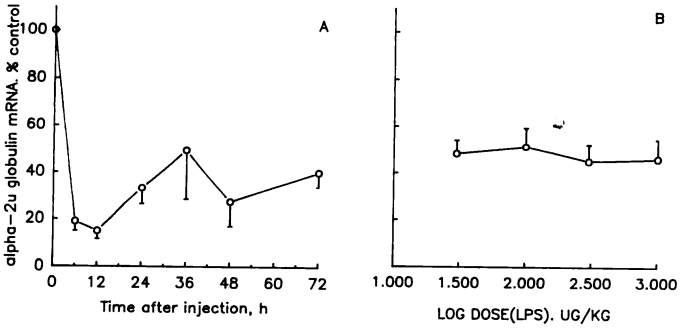

Fig. 4. Time course (A) and dose dependence (B) of the repression of α2u-globulin mRNA by LPS in male rat liver. In A, rats were administered a 1 mg/kg dose of LPS and killed at the indicated times. The number of rats in each group is given in Table 1. In B, rats were administered the indicated dose of LPS and killed 24 hr later. The number of rats in each group is given in Table 3. Relative levels of mRNA in the livers were determined and normalized to the hepatic content of β-actin mRNA, as described in the text, and are expressed as percentages of the mean value for the control male group.

livers of the LPS-treated rats were not increased above control male levels (not shown). A small increase in P-450i protein expression was detected 24 hr after LPS in one experiment but was not observed in a second experiment (not shown).

In order to establish that a typical acute phase response was being elicited in our experimental model, I examined the expression of mRNA for the negative acute phase protein α2u-globulin in the same livers. This protein was chosen not only because it is repressed by endotoxin administration (20) but also because it shares some regulatory aspects with P-450h; it is expressed in a male-specific fashion in rat liver and is regulated by androgens and by somatotropin (36). Fig. 4A shows that LPS produced a decrease in hepatic α2u-globulin mRNA, with a time course that was similar to that observed for P-450h mRNA.

Time course of LPS effects in female rat liver. LPS administration to female rats suppressed microsomal levels of total P-450 and P-450i apoprotein. However, in female rats a maximal decrease (29%) in total microsomal P-450 content occurred only 12 hr after LPS administration (Table 2), compared with 24 hr in males. In females, the mean hepatic microsomal contents were also lowered by LPS treatment (Table 2), so that total P-450 content/g of liver was decreased by 56% after LPS administration. As observed in the male, endotoxin caused decreases in the cytochrome b_5 content and NADPH-cytochrome *c* reductase activities of the microsomes. These decreases also occurred more rapidly in female rats than in males. Thus, microsomal cytochrome b_5 levels were reduced by 25% after only 6 hr and were maximally suppressed (32% decrease) by 12 hr (Table 2). NADPH-cytochrome *c* reductase activities were also maximally decreased (33%) after only 12 hr, whereas in the male the activity was unaffected at that time (Tables 1 and 2).

The more rapid decrease in total P-450 levels in female rats compared with males was also reflected in the kinetics of the suppression of P-450i. A decrease in the microsomal P-450i content was observable within 6 hr of LPS injection (Figs. 5 and 6). P-450i expression reached a minimum of 27% of control levels after 12 hr (17% of control if expressed per gram of liver) and was no longer significantly different from control levels after 72 hr (Fig. 6). P-450i expression was decreased more dramatically than were total P-450 levels (73 versus 29%, respectively, at 12 hr) (Fig. 6), indicating that P-450i is selectively suppressed relative to other P-450 isozymes. P-450i mRNA was also suppressed by the endotoxin treatment, and the levels of hepatic P-450i mRNA correlated well with the levels of P-450i protein at all time points except after 72 hr (Fig. 6). At this time, the mRNA levels had fully recovered, but the P-450i protein levels were still lower than in controls.

Interestingly, the repression of P-450i mRNA by LPS was slower in the female than was repression of P-450h mRNA in the male (Figs. 2 and 6). Only a 40% decrease in P-450i mRNA was seen 6 hr after administration. The results suggest that at least part of the suppression of P-450i expression by LPS occurs at a pretranslational level but that other mechanisms may be involved. There was no evidence for increased expression of P-450h protein or mRNA in livers of LPS-treated female rats (data not shown).

Dependence of P-450 suppression on the dose of LPS. In view of the report by Stanley *et al.* (28) that LPS suppression of constitutive P-450 isozyme expression in mice requires doses of endotoxin much higher (1 mg/kg) than those required to elicit an acute phase response of the liver, it was critical for the present study to determine whether effects on cytochrome P-450 expression in rat liver occur at lower doses of LPS. Table 3 shows that total microsomal P-450 content was decreased in the livers of male rats at the lowest dose studied (30 μg/kg) and that 100 μg/kg produced a maximal effect. The decrease in total microsomal protein was essentially maximal at 100 μg/kg endotoxin. NADPH-cytochrome *c* reductase activities of the microsomes were not significantly affected 24 hr after any dose of endotoxin, although as before there was a tendency for the mean values to diminish with LPS treatment (Table 3). Cytochrome b_5 levels were reduced to 84% of control by 100 μg/kg LPS, with a further small reduction occurring at higher doses (Table 3).

Repression of P-450h mRNA in male rat livers was essentially maximal at 30 μg/kg LPS, the lowest dose studied (Fig. 7). This was also true for repression of the negative acute phase protein α2u-globulin (Fig. 4B). P-450h protein expression was also significantly suppressed at this dose, but there was a slightly greater reduction at the higher dose of 100 μg/kg (Fig. 7). In comparison, a dose of 300 μg/kg given daily for 3 days was reported to have no effect on constitutive P-450 expression in mice (28).

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TABLE 2

Temporal dependence of the effect of LPS on hepatic microsomal protein, NADPH-cytochrome c reductase activities, and contents of cytochrome b_5 and P-450 in female rats

Experimental details are identical to those described in Table 1.

Time after injection	n	Microsomal protein	Total P-450	Cytochrome b_5	NADPH-cytochrome c reductase
hr		*mg/g of liver*	*nmol/mg*	*nmol/mg*	*nmol/mg/min*
0	4	5.4 ± 0.6	0.95 ± 0.04	0.84 ± 0.03	126 ± 5
6	4	6.1 ± 0.6	0.73 ± 0.03[a]	0.63 ± 0.02[b]	113 ± 11
12	4	3.3 ± 0.2[a]	0.67 ± 0.07[b]	0.57 ± 0.04[b]	84 ± 9[b]
24	4	4.5 ± 0.6	0.71 ± 0.07[a]	0.59 ± 0.06[b]	99 ± 9
48	4	3.8 ± 0.3	0.80 ± 0.02	0.69 ± 0.02[a]	87 ± 6[a]
72	4	3.8 ± 0.6	0.93 ± 0.18	0.69 ± 0.04[a]	85 ± 3[b]

[a] Significantly different from control group, $p < 0.05$.
[b] Significantly different from control group, $p < 0.01$.

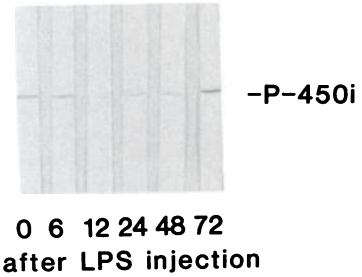

Fig. 5. Western blot showing repression of P-450i expression in female rat liver by endotoxin. Five micrograms of hepatic microsomal protein from control rats or rats killed at the indicated time after a 1 mg/kg injection of LPS were subjected to polyacrylamide gel electrophoresis in the presence of sodium lauryl sulfate and were blotted on a nitrocellulose filter. The filters were probed with antibodies to P-450i as described in the text. The figure is a composite of nonadjacent lanes taken from a single blotting experiment.

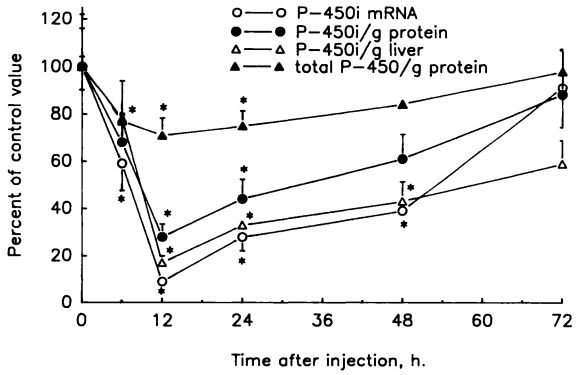

Fig. 6. Time course of repression of P-450i expression in female rat livers by endotoxin. Rats were given a single intraperitoneal injection of 1 mg/kg LPS and sacrificed at the times indicated. Relative hepatic levels of total P-450, P-450i apoprotein and P-450i mRNA were determined as described in the text and as described for P-450h in Fig. 2. Values were calculated as described in Fig. 2. All values are given as percentages of the mean values for the control female group. The number of animals in each group is given in Table 2. * Significantly different from control, $p < 0.05$.

Effect of local inflammation on P-450 expression in male rats. Both local inflammation and endotoxin provoke the hepatic acute phase response. Experimentally induced inflammatory responses have also been reported to reduce microsomal P-450 concentrations and drug-metabolizing activities in the liver (10, 12–14). Therefore, I investigated whether the changes in P-450 isozyme expression observed after endotoxin administration would also be produced by inflammation.

Inflammation was induced in rats by the subcutaneous injection of turpentine, a well characterized model for studying the acute phase response (18, 20). The effects on microsomal hepatic expression, as well as total P-450, cytochrome b_5, and NADPH-cytochrome *c* reductase are summarized in Table 4. Experimental inflammation produced a more profound decrease in microsomal P-450 (54% after 40 hr) than did endotoxin. However, in contrast to the endotoxin effects, microsomal protein content was actually increased in the inflammation model (47% increase after 40 hr). Despite this fact, the P-450 content/g of liver was still decreased by 32%, relative to control, 40 hr after treatment (Table 4). The effects of inflammation on microsomal cytochrome b_5 content were similar to those of endotoxin, with a 35% decrease in cytochrome b_5 content occurring after 40 hr. Unlike the effect of endotoxin, however, NADPH-cytochrome *c* reductase activities of the microsomes were unaffected in the inflammation model (Table 4).

The effects of experimental inflammation on P-450h expression were also qualitatively similar to those of endotoxin. That is, P-450h mRNA was reduced to 5% of control levels, and the effect on the mRNA was more rapid than the effect on the P-450h apoprotein (Table 4). Total hepatic P-450h protein was less affected by inflammation than was the mRNA. However, compared with the endotoxin model, suppression of P-450h protein expression by inflammation was of slower onset but of greater magnitude (cf. Table 4 and Fig. 2). Repression of α2u-globulin mRNA levels was also of greater magnitude in the turpentine-treated rats, compared with those treated with LPS.

Discussion

The results presented herein clearly demonstrate that two major constitutive hepatic isozymes of cytochrome P-450 are suppressed during the acute phase response to endotoxin and that this suppression is at least partially due to pretranslational mechanisms in both cases. Because microsomal levels of P-450h and P-450i can account for up to half of the spectrally detectable P-450 in males and females, respectively (4, 5), this suppression presumably makes a significant contribution to the decreases in total microsomal P-450 observed after endotoxin treatment.

Microsomal levels of P-450i were more affected by endotoxin than were total P-450 levels in female rat livers (70% decrease versus 30%) (Fig. 6), i.e., P-450i expression was specifically

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TABLE 3

Dose dependence of the effect of LPS on hepatic microsomal protein yield, NADPH-cytochrome c reductase activities, and contents of cytochrome b_5 and P-450 in male rats

Rats were given a single intraperitoneal injection of the indicated dose of LPS and were sacrificed 24 hr later. Other experimental details are identical to those described in Table 1.

Dose of LPS	n	Microsomal protein	Total P-450	Cytochrome b_5	NADPH-cytochrome c reductase
μg/kg		mg/g of liver	nmol/mg	nmol/mg	nmol/mg/min
0	4	5.6 ± 0.2	1.07 ± 0.02	0.94 ± 0.03	139 ± 7
30	4	4.9 ± 0.4	0.91 ± 0.05[a]	0.81 ± 0.06	133 ± 4
100	4	4.2 ± 0.3[a]	0.80 ± 0.03[b]	0.79 ± 0.03[a]	107 ± 7
300	3	3.9 ± 0.5[b]	0.84 ± 0.05[b]	0.76 ± 0.03[a]	109 ± 17
1000	4	4.0 ± 0.3[b]	0.85 ± 0.03[b]	0.72 ± 0.02[b]	114 ± 8

[a] Significantly different from control group, $p < 0.05$.
[b] Significantly different from control group, $p < 0.01$.

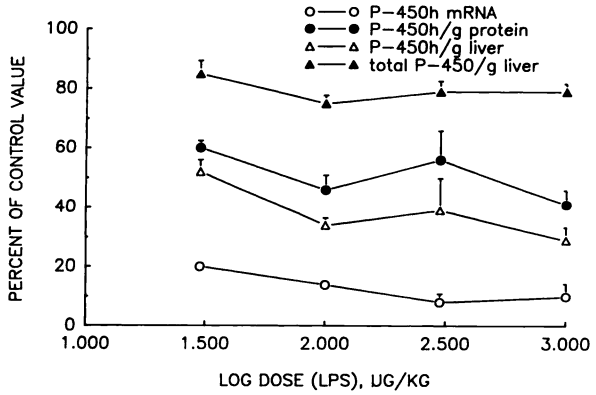

Fig. 7. Dose dependence of the suppression of P-450h by LPS in male rats. Male rats were injected intraperitoneally with the indicated dose of LPS and were killed 24 hr later. The number of animals in each group is given in Table 3. Measurements and calculations were performed as described in Fig. 2. All points on this graph were significantly different from control, $p < 0.05$.

TABLE 4

Effect of turpentine-induced inflammation on hepatic microsomal proteins and P-450h expression in male rats

Rats were injected subcutaneously with 5 ml/kg turpentine and were sacrificed at the times indicated. Relative levels of P-450h protein, P-450h mRNA, and α2u-globulin mRNA are expressed as percentage of the control male level. Other details are as described in Table 1.

Treatment group	Control	Turpentine, 24 hr	Turpentine, 40 hr
n	4	4	3
Liver weight (% of body weight)	4.8 ± 0.2	4.7 ± 0.2	5.2 ± 0.1
Microsomal protein (mg/g of liver)	5.3 ± 0.6	6.9 ± 0.2	7.8 ± 0.1[a]
Total P-450			
(nmol/mg)	1.17 ± 0.05	0.67 ± 0.05[a]	0.54 ± 0.02[a]
(nmol/g liver)	6.13 ± 0.58	4.61 ± 0.36	4.20 ± 0.16[b]
P-450h protein			
(mg protein)	100 ± 11	63 ± 7[b]	21 ± 2[b]
(g liver)	100 ± 18	81 ± 10	30 ± 3[b]
P-450h mRNA	100 ± 13	4 ± 1[a]	5 ± 1[a]
α2u-Globulin mRNA	100 ± 28	3 ± 1[a]	3 ± 1[a]
NADPH-cytochrome c reductase (nmol/min/mg of protein)	138 ± 8	145 ± 7	150 ± 1
Cytochrome b_5 (nmol/mg)	0.57 ± 0.05	0.46 ± 0.02	0.37 ± 0.05[a]

[a] Significantly different from control, $p < 0.01$.
[b] Significantly different from control, $p < 0.05$.

suppressed relative to other P-450 isozymes. Assuming that P-450i constitutes 25 to 50% of the total P-450 in microsomes of normal females (see above), the decrease in P-450i content could account for 58–116% of the observed decrease in spectrally measurable P-450. Specific suppression of P-450h levels in male rats relative to total P-450 was less evident at earlier time points. However, at 72 hr after treatment microsomal P-450 levels were decreased by 48% and total P-450 levels were decreased by 18%. Again, if P-450h accounts for 25 to 50% of the spectrally measurable P-450 in the microsomes, P-450h could be responsible for 67–133% of the decrease in total P-450. The preceding estimations do not account for the possibility that a significant fraction of each microsomal P-450 isozyme lacks prosthetic heme. In addition, the immunological assays may overestimate the absolute contents of P-450 isozymes in the microsomes. However, these calculations serve to illustrate the potential importance of suppression of these constitutive isozymes in the decrease in total microsomal P-450 that occurs after endotoxin treatment.

Endotoxin administration caused a rapid decrease in the hepatic levels of P-450h mRNA in male rats (almost maximal by 6 hr), which was followed by a slower decline in the levels of its cognate protein. Full suppression of the protein was observed at 24 hr. These data are consistent with the repression of P-450h mRNA being primarily responsible for the decrease in protein expression. It remains to be determined whether the decrease in P-450h mRNA is due to a decrease in P-450h gene transcription or, for instance, to increased mRNA degradation. The repression of hepatic mRNAs for some negative acute phase proteins, including α2u-globulin, has been shown to be transcriptionally controlled (20). In this regard, it is interesting that the kinetics of repression of the P-450h and α2u-globulin mRNAs are similar.

In further reference to regulation of P-450h expression, I found that the hepatic levels of P-450h protein were maximally suppressed to about 36% of control, whereas its mRNA was repressed to 10% of controls. One explanation for the lack of quantitative correlation could be that either the cDNA or the antibody to P-450h is not specific. Fig. 1 shows that the immunoabsorbed antibody used recognizes a single protein band on a Western blot of microsomes from male rat liver, and I have previously shown that no protein in female rat liver is recognized. I cannot exclude the possibility that the antibody may cross-react with another male-specific protein of the same mobility that is less affected by endotoxin. However, previous studies of P-450h regulation using this antibody have revealed

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no discrepancies. The cDNA probe used to detect P-450h mRNA in this study detects a single mRNA size class on Northern blots (34) and fails to recognize any RNA species in female rat liver. The mRNA recognized by the probe exhibits the same dependence on the growth hormone secretory pattern as does P-450h (34). Moreover, the washing conditions for the slot-blot assay used in the present study were of high stringency. Again, although cross-hybridization with a highly homologous, male-specific mRNA cannot be excluded, the data available suggest that this is unlikely.

Two other possible mechanisms to explain the relatively greater effect on P-450h mRNA than on its protein are decreased turnover of the P-450h apoprotein and an increased rate of translation of the remaining P-450h mRNA. Some drugs can cause an increased hepatic content of P-450 isozymes without an increase in their cognate mRNAs, but I am unaware of previous evidence for P-450 protein stabilization in the face of repression of the mRNA. The rat liver may be using this mechanism to help maintain the levels of an important constitutive enzyme, while at the same time diverting its transcriptional and translational machinery towards manufacture of secretory proteins.

There was no clear temporal distinction between the LPS-induced declines in the protein and mRNA for P-450i. Indeed, the relative levels of P-450i apoprotein were well correlated with those of its mRNA at all time points except at 72 hr, when levels of the mRNA recovered more rapidly than the protein. On one hand, the good correlation suggests that suppression of P-450i is pretranslationally mediated but, on the other, the fact that the protein levels declined as rapidly as the mRNA suggests that P-450i apoprotein turnover may also be increased by endotoxin. This latter explanation need not be invoked if P-450i had a rapid rate of turnover. No direct measurements of the half-lives of P-450h or P-450i are available. However, those P-450 isozymes studied so far have half-lives on the other of 20–30 hr and, because P-450i induction by growth hormone in hypophysectomized rats takes several days (37), it may be inferred to be a relatively long-lived isozyme.

Others have reported that endotoxin administration is accompanied by a decrease in the hepatic microsomal cytochrome b_5 content (28). In the present study, the mean microsomal levels of cytochrome b_5 were consistently decreased in endotoxin-treated rats, although the decreases were not always statistically significant due to the small number of rats per group. The lack of effect of endotoxin on microsomal NADPH-cytochrome *c* reductase activities in male rat livers within 24 hr of administration is also consistent with previous observations in mice (28). However, in this paper I found that the microsomal activity of this enzyme is decreased at later time points in male rats and is more rapidly decreased in female rats.

A striking feature of the effects of endotoxin on microsomal P-450, cytochrome b_5, and NADPH-cytochrome P-450 reductase levels is that they are more rapidly decreased in female rats than in males. The author is unaware of any known sex differences in rat liver metabolic pathways that might explain this observation.

A critical aspect of this study was the fact that endotoxin was able to produce a near-maximal suppression of P-450 expression at a dose of 30 μg/kg, one tenth of the dose reported to have no effect on constitutive P-450 isozymes and mRNAs in mice (28). Moreover, 30 μg/kg is in the range required to produce a maximal induction of mRNA for angiotensinogen in rat liver (21), indicating that suppression of P-450 gene expression is essentially part of the acute phase response to endotoxin. Thus, one might reasonably expect that similar effects on P-450 expression would be seen in rats with bacterial infections and that there may be specific repression of constitutive P-450 expression in patients with bacterial infections. In this regard, however, it should be stressed that humans do not possess genes corresponding to P-450h or P-450i (1).

Experimental inflammation, as well as infection, provokes an acute phase response of the liver. This study demonstrates that local inflammation induced by subcutaneous injection of turpentine produces effects on microsomal P-450 expression and cytochrome b_5 levels in male rats that are very similar to those of endotoxin. The most notable differences were that the inflammation response was somewhat slower, possibly reflecting a lag time in release of inflammatory mediators (38), and also that the responses to inflammation were of greater magnitude, especially with regard to the effect on total microsomal P-450. In addition, turpentine-induced inflammation was accompanied by an increase in microsomal protein yield, whereas endotoxin had the opposite effect. Interestingly, no effect on NADPH-cytochrome *c* reductase activities was seen within 40 hr in the rats injected with turpentine. Because the effects on P-450 expression were slower to develop in the inflammation model, one would need to examine NADPH-cytochrome *c* reductase activities at longer time intervals to be certain that there was no effect.

The humoral mediators of the induction of acute phase proteins and repression of the negative acute phase proteins by endotoxin or inflammation are thought to be the cytokines IL-1 and IL-6. Although both of these mediators have many actions on different cell types (24) and could therefore influence hepatic function by a variety of indirect mechanisms, they have been shown to mimic at least some of the aspects of the acute phase hepatic response in cultured hepatic cell systems (25, 26, 39). Current evidence favors IL-6 as the most important mediator of the hepatic response, because its spectrum of induction and repression of the acute phase and negative acute phase proteins is broader than that of IL-1 (26, 39). The negative acute phase protein α2u-globulin is a male-specific hepatic gene product and, like P-450h, is dependent upon testosterone and growth hormone for its expression (3, 4, 36). It will be interesting to test whether P-450h and P-450i expression are also suppressed by IL-1 or IL-6. In that vein, it has long been known that interferon inducers cause a decrease in hepatic P-450 content (40), and more recently it has been shown that a recombinant consensus α-interferon suppresses P-450 levels and activities when injected into hamsters (41). A pivotal question will be whether suppression of these P-450 isozymes by endotoxin is due to a specific and direct action of cytokine mediators or simply reflects the diversion of the hepatic transcriptional machinery to strongly induced secreted gene products.

In dealing with the regulation of isozymes h and i under any conditions, one must always bear in mind that the most important physiological regulator of their expression is the growth hormone secretory pattern (3–5). Because it is known that endotoxin administration causes a suppression of growth hormone secretion that lasts for at least 6 hr (42), it must be considered whether alteration of normal growth hormone con-

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trol could be partly or wholly responsible for the effects on P-450h and i expression. Experiments are now in progress to test this hypothesis.

Another possible mechanism for the repression of P-450 gene expression during the acute phase response might be an alteration in hepatic heme metabolism. Heme oxygenase is induced within a few hours of endotoxin administration (15, 43). Bissell and Hammaker (15) have suggested that the stimulation of heme oxygenase is consequent to degradation of cytochrome P-450 and subsequent liberation of heme, because an increased free hepatic heme pool induces heme oxygenase. However, others have observed induction of heme oxygenase before any detectable decrease in holocytochrome P-450 (43). Moreover, it has recently been reported that purified heme oxygenase can remove and degrade heme from purified cytochrome P-450 preparations (44). Thus, it is conceivable that induction of heme oxygenase may be a causative factor for degradation of cytochrome P-450 heme and thereby accelerates degradation of the protein moiety. Alternatively, depletion of the hepatic heme pool due to heme oxygenase induction might inhibit transcription of constitutive P-450 genes. Heme has been reported to be a positive regulator of transcription of inducible P-450 genes (45), although this observation has yet to be confirmed by other laboratories.

In conclusion, depression of hepatic cytochrome P-450 levels during the acute phase response to endotoxin is accompanied by a suppression of the protein and mRNA for the major constitutive P-450 isozymes h and i. Both pre- and posttranslational mechanisms appear to be involved in regulation of these isozymes during the acute phase response. The humoral mediators of these effects have yet to be identified.

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