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Regulation of Cytochrome P450IIC12 Expression by Interleukin-1α, Interleukin-6, and Dexamethasone

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SUMMARY

During the acute phase response to bacterial endotoxin in rats, hepatic levels of cytochrome P450IIC12 [AH, reduced flavoprotein:oxygen oxidoreductase (RH hydroxylating), EC 1.14.14.1] (P450IIC12) apoenzyme and mRNA are suppressed. We set out to determine the effects of potential humoral mediators of inflammation on the expression of P450IIC12 in female rats. A single injection of 12,000 or 60,000 units of interleukin-1α had no effect on total cytochrome P450 content or P450IIC12 mRNA measured 12 hr later, although P450IIC12 apoenzyme was slightly but significantly increased by the higher dose. In the second experiment, animals were given dexamethasone (100 μg/kg at −30 min), interleukin-1α (30,000 units/kg at 0, 2, and 4 hr), or both and were sacrificed at 12 hr. Treatment with interleukin-1α alone significantly suppressed total cytochrome P450, P450IIC12 apoenzyme, and P450IIC12 mRNA to 77, 53, and 65% of control levels, respectively; β-actin mRNA was significantly increased (206% of control levels). Treatment with dexamethasone alone suppressed total cytochrome P450 and P450IIC12 mRNA (73% of controls) but did not significantly affect P450IIC12 apoenzyme measured 12.5 hr later. Again, β-actin mRNA was increased. When both interleukin-1α and dexamethasone were given, total cytochrome P450 and P450IIC12 mRNA (43% of controls) were suppressed, and β-actin mRNA was significantly increased. In the third experiment, animals were injected at 0 and 12 hr with dexamethasone (83 μg/kg), interleukin-6 (33 μg/kg), or both. Interleukin-6 alone did not significantly affect total cytochrome P450 or P450IIC12 apoenzyme or mRNA. Dexamethasone alone suppressed P450IIC12 apoenzyme and mRNA (to 52 and 41%, respectively, of controls). Treatment with both interleukin-6 and dexamethasone significantly suppressed total cytochrome P450 and P450IIC12 apoenzyme and mRNA; suppression of P450IIC12 mRNA (to 16% of controls) was greater than with dexamethasone alone. No change in the transcription rate of *CYP2C12* was observed 24 hr after initiation of treatment with dexamethasone (83 μg/kg at 0 and 12 hr) or 12 hr after initiation of treatment with interleukin-1α (30,000 units/kg at 0, 2, and 4 hr). We conclude that, in this model, interleukin-1α and glucocorticoids are important mediators of the suppression of hepatic P450IIC12 expression during inflammation. Interleukin-6 was not as potent, but it did potentiate the effects of dexamethasone. Suppression of P450IIC12 expression by dexamethasone and interleukin-1α appeared to be mediated at a pretranslational level, but the possibility of a transcriptional effect needs to be further investigated.

P450IIC12[1] is one of two major constitutive P450s in the liver of adult female rats. The expression of this protein in normal rats is dependent on the female-specific pattern of growth hormone secretion, which consists of pulses of high frequency and low amplitude superimposed on a high basal level of plasma growth hormone (1). In contrast, the male-specific pattern of growth hormone secretion, which is characterized by pulses of high amplitude and low frequency superimposed on a low basal level of hormone, suppresses expression of P450IIC12. The female pattern of growth hormone secretion, and hence of hepatic P450IIC12 expression (1), is set during sexual differentiation of the brain in the neonatal rat. Although growth hormone is an important physiological regulator, P450IIC12 expression is also affected by pathophysiological conditions. We have previously shown that expression of P450IIC12 apoenzyme and mRNA is reduced during inflammation induced by treatment with bacterial endotoxin (2). The humoral mediators of this effect are not known.

During the acute phase response to inflammation, major changes occur in the synthesis and secretion of many hepatic proteins. Synthesis of positive acute phase proteins, such as α_2-macroglobulin, increases, whereas the synthesis of negative acute phase proteins, such as albumin, decreases (3). Cytokines are important mediators of the inflammatory response (4). In rats, IL-1 alone mimics some, but not all, of the inflammatory changes in hepatic secretory proteins and their cognate mRNAs

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[1] P450IIC12 is the product of the *CYP2C12* gene and also bears the trivial names P-450i, P-450female, P-4502d, fRLM4, and P-45015β.

ABBREVIATIONS: P450, cytochrome P-450; IL-1, interleukin-1; IL-6, interleukin-6; DEX, dexamethasone.

(5). Treatment of rats with IL-6 increases hepatic secretion of α_2-macroglobulin, fibrinogen, thiostatin, and hemopexin (6). Thus, IL-1 and IL-6 may both mediate the effects of inflammation on the expression of hepatic proteins.

Inflammation also stimulates the hypothalamic-adenophyophyseal-adrenal cortical axis (4, 7); therefore, glucocorticoids also may be mediators of the effects of inflammation on hepatic protein synthesis. Glucocorticoids are required for maximal induction of positive acute phase hepatic proteins in response to IL-6 treatment *in vivo* (6). When rat hepatocytes were exposed *in vitro* to media conditioned by squamous carcinoma COLO-16 cells or by activated monocytes, simultaneous treatment with DEX enhanced the synthesis of specific acute phase proteins (8).

The findings described above, together with the fact that glucocorticoids are well known to affect P450 gene expression (9, 10), suggest that these adrenal hormones and/or cytokines associated with inflammation may contribute to suppression of P450 in inflammatory reactions. IL-1 suppresses total hepatic P450 content and activity in mice *in vivo* (11–13) and *in vitro* (11, 13). The hepatic IL-1 receptor is required to mediate IL-1 suppression of total P450 content in cultured hepatocytes (14). However, these studies did not look at specific isozymes of P450.

The purpose of the present experiments was to determine whether IL-1 or IL-6 alone or in combination with DEX, a synthetic glucocorticoid, could mimic the suppression of P450IIC12 induced by bacterial endotoxin or local inflammation. In addition, we used a nuclear run-on assay to determine whether suppression occurred at the level of *CYP2C12* gene transcription.

Materials and Methods

Cytokines. Recombinant human IL-1α (2×10^4 units/μg) was generously provided by Dr. P. L. Simon, Smith Kline & French Laboratories (Swedeland, PA). Recombinant human IL-6 (batch 1406-131; 4×10^6 units/mg) was a gift of Dr. G. Wong, Genetics Institute (Cambridge, MA).

Animals and treatments. Female Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN), 6–7 weeks old, were housed using a 12-hr light cycle and were allowed access to food and water *ad libitum*. Animals were sacrificed by CO_2 asphyxiation.

IL-1 was diluted in 10 mM sodium phosphate buffer, pH 7.4, 0.15 M NaCl, 0.1% bovine serum albumin, and injected intraperitoneally. IL-6 was prepared in 10 mM sodium phosphate buffer, pH 7.4, 0.15 M NaCl, and injected intraperitoneally. DEX (Sigma Chemical Co., St. Louis, MO) was suspended in corn oil and injected subcutaneously. Control animals received the appropriate vehicle(s).

Hepatic nuclei, microsomes, and RNA. Livers were rapidly excised and perfused with cold 1.15% KCl. Nuclei were prepared by pelleting through sucrose (15). Microsomes were isolated by differential centrifugation and washed with pyrophosphate buffer (16). Total RNA was prepared by the method of Chomczynski and Sacchi (17). Poly(A)$^+$ RNA was purified from total RNA by batch absorption with oligo(dT)-cellulose.

Microsomal protein and Western blot assays. Total microsomal protein was determined as described by Lowry *et al.* (18). Total microsomal P450 content was determined from the CO difference spectrum of the reduced protein (19); values were expressed as nmol of P450/mg of microsomal protein.

Relative levels of P450IIC12 apoenzyme in microsomal preparations were measured by Western blot assay. Microsomal proteins were separated by polyacrylamide (7.5%) gel electrophoresis, in the presence of sodium dodecyl sulfate, and were transferred to nitrocellulose filters essentially as described by Towbin *et al.* (20). The filters were blocked, probed, and developed as described previously, using a monoclonal antibody specific for P450IIC12 (21). The intensities of the bands were quantified using an LKB Ultroscan laser densitometer. The amount of protein applied to the gel was adjusted such that there was a linear relationship between protein and band intensity for all samples. Band intensities were expressed per mg of microsomal protein, and the values for individual livers were expressed as a percentage of the mean value for the control group.

RNA slot blots and Northern blots. The relative levels of P450IIC12, albumin, and β-actin mRNA in total hepatic RNA [and, in some experiments, poly(A)$^+$ RNA] were quantified by slot blot assay (22), as described previously (2), except that the high stringency wash for albumin was at 66°. Albumin mRNA was used as a control for the negative acute phase proteins; levels of albumin mRNA have been shown to be suppressed by IL-1, IL-6, and inflammation (5, 6, 23). Cloned cDNA C-6 (24) was used to probe for P450IIC12 mRNA; albumin cDNA was kindly provided by Dr. Gunnar Norstedt of the Karolinska Institute. β-Actin mRNA was detected with a full-length cDNA probe (25). The intensities of the bands were quantified by laser densitometry. As above, care was taken to ensure that the densitometric signal was proportional to the amount of RNA applied to the filter. All slot blot assay results were corrected for the relative content of poly(A)$^+$ RNA in each sample, which was estimated by probing the slot blots with an oligo(dT) probe, as described below.

Relative contents of poly(A)$^+$ RNA in the total RNA samples used for the slot blots described above were measured by probing of identical blots with 5′-^{32}P-labeled oligo(dT)$_{16}$. To each slot blot, we applied 0.5, 1, and 2 μg of 20 different total RNA samples. Twenty picomoles of 5′-hydroxy(dT)$_{16}$ (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) were labeled with polynucleotide kinase and hybridized to the immobilized RNA, at a concentration of 1 pmol/ml, for 16 hr in 6× SSPE (60 mM sodium phosphate buffer, pH 7.7, containing 1.08 M NaCl and 6 mM EDTA) at room temperature. The blots were washed three times with 6× standard saline citrate (150 mM sodium citrate buffer, pH 7.0, 0.9 M NaCl), containing 0.1% sodium dodecyl sulfate, for 5 min each time at room temperature, dried, and subjected to autoradiography and densitometry as described above.

Northern analyses of total hepatic RNA were performed essentially as described by Sambrook *et al.* (26). Briefly, hepatic RNA was denatured and subjected to agarose gel electrophoresis (1% agarose) in the presence of formaldehyde. The RNA was blotted from the gels onto Magnagraph nylon filters (Micron Separations Inc., Westboro, MA) by capillary action and was fixed by UV irradiation. Blots were hybridized with the cDNA probes, washed, and subjected to autoradiography.

Transcription run-on assays. Transcription run-on assays were performed by a modification (27) of the methods of Birch and Schreiber (28) and Marzluff and Huang (29), using the cDNA probes referenced above.

Statistical analysis. Densitometric data from Western blot assays, slot blot assays, and transcription run-on assays were expressed as percentages of the mean of the control group. One-way analysis of variance and Dunnett's test were used to determine differences among treatment groups. Bartlett's test for equivalence of variance was used, and logarithmic transformations were done when necessary. Differences were considered to be significant if p was <0.05. Data are presented in the figures as means ± standard errors.

Results

Modulation of hepatic expression of β-actin mRNA and use of oligo(dT) slot blots for determination of mRNA contents of samples. Normalization to β-actin, as an mRNA that is thought to be expressed at constant levels, is a widely used and accepted method to correct for possible artifacts resulting from the use of total RNA in Northern and slot blot assays. As will be seen below, we found that actin mRNA

levels were significantly elevated in the livers of animals from some of the treatment groups. Therefore, actin mRNA could not be used as a control.

Errors in the estimated concentration of RNA in total RNA preparations may arise from contamination of the samples with species that absorb at 260 nm or from nonuniform loss of material during gel loading or blotting procedures. In the case of the slot blot assays used in this study, the latter problem may be dismissed, because the RNA is applied directly to the membrane. We addressed the problem of accurately estimating the relative mRNA contents of the total RNA samples by probing slot blots with a radiolabeled oligo(dT)$_{16}$ probe. Using this assay, we found no significant differences among any of the groups in the poly(A)$^+$ RNA contents of the total RNA samples (not shown). All mRNA quantitations presented here have been corrected for poly(A)$^+$ RNA content determined by this assay.

Effects of IL-1α and DEX on P450IIC12 protein and mRNA levels. In the first experiment with IL-1, the cytokine was given alone, as a single injection of either 12,000 ($n = 6$) or 60,000 units/kg of body weight ($n = 5$; control, $n = 5$); the animals were sacrificed at 12 hr. With this treatment protocol, neither dose of IL-1 significantly affected levels of mRNA for P450IIC12, β-actin, or albumin (not shown); immunoreactive P450IIC12 apoenzyme was slightly (130 ± 10% of control levels) but significantly increased by the higher dose. The lack of suppression by IL-1, in contrast to the response seen in the subsequent experiments, agrees with the findings of others that multiple injections of IL-1 are more effective than a single injection in mimicking the effects of inflammation on hepatic gene products (5). This may be due to a short biological half-life of the cytokines (6).

We then tested the effects of multiple injections of IL-1 with or without glucocorticoid. DEX (100 μg/kg of body weight) or vehicle was injected at −30 min; IL-1 (30,000 units/kg of body weight) or vehicle was injected at 0, 2, and 4 hr, and the animals were sacrificed at 12 hr. Each group contained five animals. With this treatment protocol, IL-1 significantly suppressed total P450 (to 77% of the control group; Fig. 1), P450IIC12 apoenzyme (53% of controls; Figs. 2 and 3) and mRNA (65% of controls; Figs. 2 and 4), and albumin mRNA (56% of controls; Fig. 1). The amount of β-actin mRNA was significantly increased (to 206% of controls; Figs. 1 and 4). Similar effects of IL-1 on P450IIC12 mRNA and β-actin mRNA were seen when the slot blot assays were repeated using purified poly(A)$^+$ RNA (data not shown).

Treatment of animals with a single dose of DEX (100 μg/kg) significantly suppressed total P450 12.5 hr later (to 80% of the control group; Fig. 1) but did not affect albumin mRNA (Fig. 1) or P450IIC12 apoenzyme (Fig. 2). However, P450IIC12 mRNA was suppressed to 73% of control levels (Figs. 2 and 4), whereas β-actin mRNA levels were elevated to 163% of controls (Figs. 1 and 4).

When IL-1 was administered in addition to DEX, P450IIC12 mRNA was suppressed (to 43% of the control group; Figs. 2 and 4) but the apoenzyme was not (83% of controls; Fig. 2). Levels of total P450, albumin mRNA, and β-actin mRNA were not significantly different from those seen when IL-1 was given alone (Fig. 1).

Effects of IL-6 and DEX on P450IIC12 protein and mRNA levels. We next investigated the effects of IL-6, alone or in combination with DEX, on P450IIC12 expression. The animals received vehicle, DEX (83 μg/kg of body weight), IL-6 (33 μg/kg of body weight), or DEX and IL-6 at 0 and 12 hr and were sacrificed at 24 hr. Each group contained five animals. Treatment with IL-6 alone significantly suppressed only albumin mRNA (to 60% of the control group; Fig. 5). Total P450, P450IIC12 apoenzyme, and P450IIC12 mRNA were not signif-

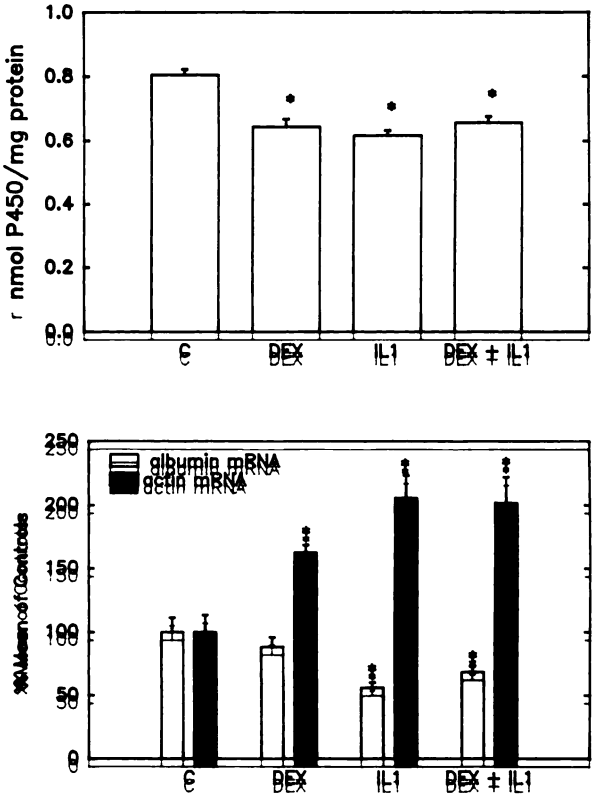


Fig. 1. Effect of treatment *in vivo* with DEX, IL-1, or DEX and IL-1 on total microsomal P450 (*upper*) and albumin and actin mRNAs (*lower*) in female rat livers. Rats were injected at $t = -0.5$ hr with either DEX (100 μg/kg, subcutaneously) or vehicle. IL-1-treated rats then received IL-1α (30,000 units/kg, intraperitoneally) at $t = 0$, 2, and 4 hr, and all animals were sacrificed at 12 hr. Total microsomal P450 and levels of albumin and β-actin mRNAs were measured as described in the text. *, Significantly different from control ($p \leq 0.05$). *C*, control.

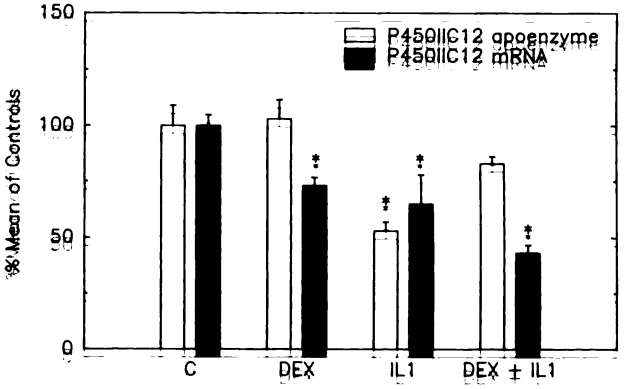


Fig. 2. Effect of treatment *in vivo* with DEX, IL-1, or both on hepatic expression of P450IIC12 apoenzyme and mRNA in female rats. Rats were treated as described in the legend to Fig. 1. Relative levels of β-P450IIC12 mRNAs were determined in total hepatic RNA as described in the text. *, Significantly different from control ($p \leq 0.05$). *C*, control.

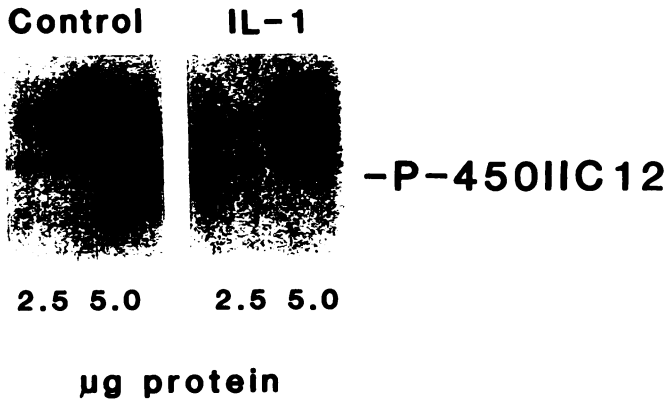


Fig. 3. Representative Western blot showing suppression of hepatic P450IIC12 apoenzyme in female rats by treatment with IL-1. Microsomal proteins from representative untreated or IL-1-treated rats (see Fig. 1 legend for treatment) were subjected to polyacrylamide gel electrophoresis, in the presence of sodium dodecyl sulfate, and blotted on a nitrocellulose filter. The filters were probed with antibodies to P450IIC12. The figure is a composite of nonadjacent lanes taken from a single filter.

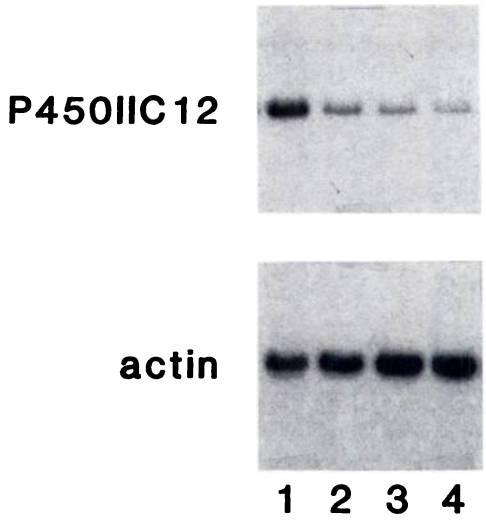
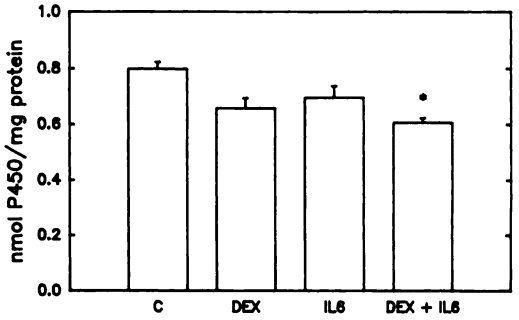


Fig. 4. Northern blot showing effects of IL-1, DEX, or both on expression of P450IIC12 and β-actin mRNA in female rat livers. Rats were treated as described in the legend to Fig. 1. Total RNA samples were subjected to agarose gel electrophoresis, blotted on nylon membranes, and probed with P450IIC12 (*upper*) or β-actin (*lower*) cDNAs, as described in the text. Each probe hybridized to a single size class of mRNA, at about 1800–2000 base pairs. The figure shows blots for hepatic RNA from a single rat in each treatment group. *Lane 1*, control; *lane 2*, DEX; *lane 3*, IL-1; *lane 4*, IL-1 plus DEX. Quantitative data summarizing RNA slot blot assays for all rats in the experiment are shown in Fig. 2.



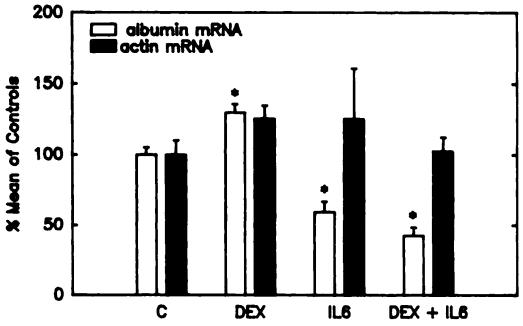


Fig. 5. Effect of treatment *in vivo* with DEX, IL-6, or both on total microsomal P450 (*upper*) and albumin and actin mRNAs (*lower*) in female rat livers. Rats received DEX (83 μg/kg), IL-6 (33 μg/kg), or both at t = 0 and 12 hr. Control (*C*) animals received vehicle only, and all rats were sacrificed at 24 hr. Total microsomal P450 and levels of albumin and β-actin mRNAs were measured as described in the text. *, Significantly different from control, ($p < 0.05$).

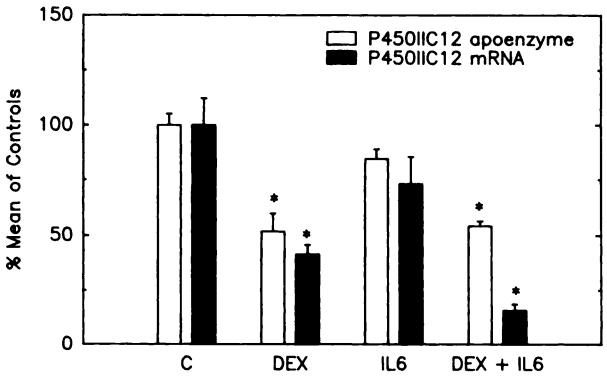


Fig. 6. Effect of treatment *in vivo* with DEX, IL-6, or both on hepatic expression of P450IIC12 apoenzyme and mRNA in female rats. Rats were treated as described in Fig. 5. Relative levels of P-450IIC12 mRNA and apoenzyme were determined as described in the text. *, Significantly different from control ($p < 0.05$). *C*, control.

icantly different from control levels (Figs. 5 and 6). β-Actin mRNA was not affected (Fig. 5).

When DEX was administered in two doses of 83 μg/kg at 0 and 12 hr, P450IIC12 apoenzyme and mRNA were suppressed to 52% and 41% of the control group, respectively (Fig. 6). Total P450 and β-actin mRNA were not significantly affected by DEX in this protocol (Fig. 5). However, albumin mRNA was significantly elevated (130%) in the livers of these animals.

Treatment with both IL-6 and DEX suppressed P450IIC12 mRNA to a significantly greater extent than DEX alone (16% of controls; Fig. 6). DEX also potentiated the suppression of albumin mRNA by IL-6 (60% to 43%), although this difference was not statistically significant (Fig. 5). The levels of P450IIC12 apoenzyme were not different from those seen after treatment with DEX alone.

Does DEX or IL-1 treatment affect the transcription rate of *CYP2C12*? The previous experiments showed that P450IIC12 mRNA was suppressed by DEX. We then tested whether this suppression could be attributed to changes in transcription rates. As before, animals (n = 3 for each group) were injected with 83 μg/kg DEX at 0 and 12 hr and sacrificed at 24 hr. Transcription rates of *CYP2C12* in animals treated with DEX were not different (136 ± 80%) from those in controls (Fig. 7), although P450IIC12 mRNA in this experiment was reduced to 20% of controls by DEX (not shown).

In the second experiment described above, IL-1 alone pro-

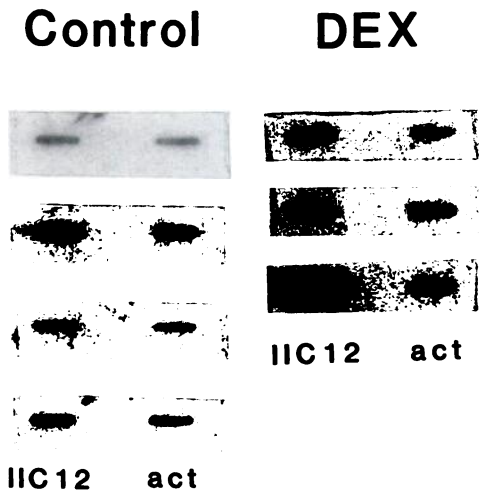


Fig. 7. Transcription run-on assays for *CYP2C12* and β-actin in nuclei isolated from livers of female rats treated with DEX. Animals were treated as described for Fig. 5. Nuclear RNA was isolated and hybridized to filters bearing linearized plasmids containing the P450IIC12 (*IIC12*) and β-actin (*act*) cDNAs (10 and 5 μg, respectively). Conditions were selected in which the autoradiographic signal was proportional to the amount of input nuclear RNA. Each filter represents nuclei from a different rat liver.

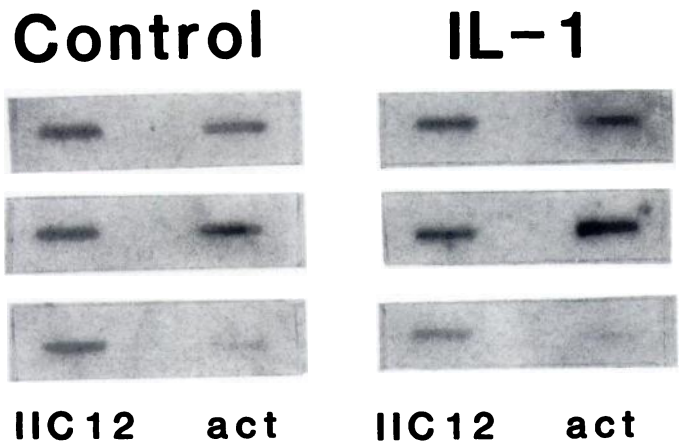


Fig. 8. Transcription run-on assays for *CYP2C12* and β-actin in nuclei isolated from livers of female rats treated with IL-1. Animals were treated with IL-1 alone, as described in the legend to Fig. 1. Hepatic nuclei were isolated and transcription run-on assays were performed as described in the legend to Fig. 7. *IIC12*, P450IIC12; *act*, actin.

duced a significant suppression of P450IIC12 apoenzyme and mRNA (to 53% and 65% of control levels, respectively). We, therefore, decided to repeat this experiment and to look for a change in the rate of transcription of the gene. Again, treatment with IL-1 (30,000 units/kg at 0, 2, and 4 hr, $n = 3$) significantly suppressed P450IIC12 apoenzyme measured at 12 hr (71% of controls). The mean level of P450IIC12 mRNA was decreased to 74% of controls, but the change was not significant (not shown). A run-on assay showed no change in the transcription rate of *CYP2C12* 12 hr after treatment (101% of controls; Fig. 8).

Discussion

Our results suggest that glucocorticoids and IL-1 are important mediators of the suppression of hepatic P450IIC12 expression during an acute inflammatory response. IL-6 alone at the dose used in this study was not as efficacious as DEX or IL-1, but it did potentiate the effects of DEX on P450IIC12 mRNA. In contrast, both IL-1 and IL-6 suppressed albumin mRNA, showing that the IL-6 treatment regimen that we used was effective in suppressing the expression of a negative acute phase protein. Clearly, there are differences in regulation of expression of the *CYP2C12* and albumin genes, with respect to their sensitivities to DEX and IL-6 suppression, or in the time courses of these responses. Our findings agree with those of Marinkovic *et al.* (6) and Baumann *et al.* (8) that DEX potentiates the effects of IL-6 on hepatic albumin expression.

The treatment protocols that we used in this study were chosen because they have been shown *in vivo* to produce effects on hepatic acute phase proteins that are similar to those seen during inflammation (5, 6). P450IIC12 levels are maximally suppressed at 12 and 24 hr after treatment of female rats with endotoxin (2). We chose 12- and 24-hr time points for the IL-1 and IL-6 experiments, respectively, based on the studies of the acute phase secretory proteins. Therefore, the effects we have observed here may underestimate the efficacies of these agents in suppressing P450IIC12, if the dose- and time-response relationships for P450IIC12 are different from those for the acute phase proteins.

The experimental treatments generally affected P450IIC12 apoenzyme and mRNA in a similar manner (e.g., treatment with IL-1 or with DEX for 24 hr). The correlation between mRNA and protein levels was better for some groups than for others, but in every case the relative levels of the mRNA were lower than or equal to those of the enzyme, suggesting that the primary effects are probably pretranslational. Detailed time-course studies should clarify the relationships between the mRNA and the apoenzyme in this model.

Injections of DEX (83 μg/kg of body weight) at 0 and 12 hr, followed by sacrifice at 24 hr, resulted in a more profound suppression of P450IIC12 mRNA and apoenzyme than did a single injection (100 μg/kg of body weight) and sacrifice at 12 hr. This difference could be due to either the longer time or the overall higher amount of DEX given in the 24-hr experiment. The doses of DEX we have used in these experiments were generally 2 orders of magnitude less than those used by most investigators studying the regulation of drug metabolism or individual P450 isozymes by glucocorticoids (9, 10). The fact that we obtained significant effects on P450IIC12 mRNA and apoenzyme with these relatively low doses of DEX lends support to the concept that physiological changes in glucocorticoids may play an important role in regulating the expression of P450IIC12.

We were unable to demonstrate a transcriptional effect of DEX or IL-1 on *CYP2C12* gene expression, in spite of the suppression of P450IIC12 mRNA and apoenzyme. We have previously shown that induction of a local inflammatory response by turpentine also suppresses P450IIC12 mRNA and apoenzyme but does not appear to affect transcription of the *CYP2C12* gene (27). However, our data do not exclude the possibility that suppression of transcription occurs at an earlier time after treatment and is transient. An extended time-course study would address this question. In addition, we and others (27, 30) have not been able to demonstrate complete sex specificity of transcription of this gene, although the level of P450IIC12 mRNA in male rats is less than 5% of that in female rats. Thus, although the data indicate that expression of this

protein may be regulated at a posttranscriptional level, a methodological problem, such as cross-hybridization of the cDNA probe to another nuclear mRNA (27), cannot be excluded.

In interpreting the data presented herein, we must emphasize that growth hormone plays a major physiological role in regulating the expression of P450IIC12. Treatment of male rats *in vivo* with endotoxin initially suppresses secretion of growth hormone (31). Both IL-6 (32) and IL-1 (33) stimulate secretion of growth hormone by isolated pituitary cells *in vitro*. Glucocorticoids can inhibit pituitary secretion of growth hormone by an effect on hypothalamic somatostatin release (34). Although IL-1 has been shown to directly suppress the amount and activity of P450 in cultured hepatocytes (11, 13, 14), changes in growth hormone secretion may play a role in the suppression of P450IIC12 we have observed in our *in vivo* experiments.

The level of β-actin mRNA in female rats was increased 12 hr after treatment with IL-1, DEX, or IL-1 plus DEX, when either total RNA or poly(A)$^+$ RNA was used in the slot blot assay. Northern blot analysis of the total RNA yielded a single distinct actin mRNA band and no evidence that the actin mRNA was selectively degraded in any of the samples. Interestingly, we have also observed an apparent induction of β-actin in female rats by treatment with an interferon inducer.[2] Surprisingly, we did not find a similar affect of IL-1 on β-actin expression in male rats.[3] Our data support those of other investigators suggesting that β-actin expression may be affected by some experimental protocols (for example, Ref. 35). Thus, β-actin is not an appropriate control in all experimental designs.

In conclusion, these experiments indicate that both IL-1 and DEX, and possibly IL-6, can mimic the suppression of P450IIC12 that occurs during an inflammatory response. When the three agents were compared at the doses used in this study, IL-1 produced the most rapid suppression and, therefore, may be the factor that initially suppresses expression of P450IIC12, whereas glucocorticoids may begin to contribute to the effects seen at later time points, because we only saw a significant effect of DEX on P450IIC12 apoenzyme after 24 hr. It is possible, however, that earlier effects of glucocorticoids might be observed if higher doses were used.

Acknowledgments

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References

1. Morgan, E. T., and J.-Å. Gustafsson. Sex-specific isozymes of cytochrome P-450. *Steroids* **49**:213–245 (1987).
2. Morgan, E. T. Suppression of constitutive cytochrome P-450 gene expression in livers of rats undergoing an acute phase response to endotoxin. *Mol. Pharmacol.* **36**:699–707 (1989).
3. Kushner, I. The phenomenon of the acute phase response. *Ann. N. Y. Acad. Sci.* **389**:39–48 (1982).
4. Arai, K.-I., F. Lee, A. Miyajima, S. Miyatake, N. Arai, and T. Yokota. Cytokines: coordinators of immune and inflammatory responses. *Annu. Rev. Biochem.* **59**:783–836 (1990).
5. de Jong, F. A., H. E. Birch, and G. Schreiber. Effect of recombinant interleukin-1 on mRNA levels in rat liver. *Inflammation* **12**:613–617 (1988).
6. Marinkovic, S., G. P. Jahreis, G. G. Wong, and H. Baumann. IL-6 modulates the synthesis of a specific set of acute phase plasma proteins *in vivo*. *J. Immunol.* **142**:808–812 (1989).
7. Rivier, C., W. Vale, and M. Brown. In the rat, interleukin-1α and -β stimulate adrenocorticotropin and catecholamine release. *Endocrinology* **125**:3096–3102 (1989).
8. Baumann, H., G. P. Jahreis, D. N. Sauder, and A. Koj. Human keratinocytes and monocytes release factors which regulate the synthesis of major acute phase plasma proteins in hepatic cells from man, rat, and mouse. *J. Biol. Chem.* **259**:7331–7342 (1984).
9. Shimada, M., N. Murayama, K. Yamauchi, Y. Yamazoe, and R. Kato. Suppression in the expression of a male-specific cytochrome P-450, P-450 male: difference in the effect of chemical inducers on P-450-male mRNA and protein in rat livers. *Arch. Biochem. Biophys.* **270**:578–587 (1989).
10. Meehan, R. R., L. M. Forrester, K. Stevenson, N. D. Hastie, A. Buchmann, H. W. Kunz, and C. R. Wolf. Regulation of phenobarbital-inducible cytochrome P-450s in rat and mouse liver following dexamethasone administration and hypophysectomy. *Biochem. J.* **254**:789–797 (1988).
11. Ghezzi, P., B. Saccardo, P. Villa, V. Rossi, M. Bianchi, and C. A. Dinarello. Role of interleukin-1 in the depression of liver drug metabolism by endotoxin. *Infect. Immun.* **54**:837–840 (1986).
12. Shedlofsky, S. I., A. T. Swim, J. M. Robinson, V. S. Gallicchio, D. A. Cohen, and C. J. McClain. Interleukin-1 (IL-1) depresses cytochrome P450 levels and activities in mice. *Life Sci.* **40**:2331–2336 (1987).
13. Bertini, R., M. Bianchi, A. Erroi, P. Villa, and P. Ghezzi. Dexamethasone modulation of *in vivo* effects of endotoxin, tumor necrosis factor, and interleukin-1 on liver cytochrome P-450, plasma fibrinogen, and serum iron. *J. Leukocyte Biol.* **46**:254–262 (1989).
14. Sujita, K., F. Okuno, Y. Tanaka, Y. Hirano, Y. Inamoto, S. Eto, and M. Arai. Effect of interleukin 1 (IL-1) on the levels of cytochrome P-450 involving IL-1 receptor on the isolated hepatocytes of rat. *Biochem. Biophys. Res. Commun.* **168**:1217–1222 (1990).
15. Blobel, G., and V. R. Potter. Nuclei from rat liver: isolation method that combines purity with high yield. *Science (Washington D. C.)* **154**:1662–1665 (1966).
16. Haugen, D. A., and M. J. Coon. Properties of electrophoretically homogeneous phenobarbital-inducible and β-naphthoflavone-inducible forms of liver microsomal cytochrome P-450. *J. Biol. Chem.* **251**:7929–7939 (1976).
17. Chomczynski, P., and N. Sacchi. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156–159 (1987).
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
19. Omura, T., and R. Sato. The carbon monoxide-binding pigment of liver microsomes. 1. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239**:2370–2378 (1964).
20. Towbin, H., T. Staehlin, and J. Gordon. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. *Proc. Natl. Acad. Sci. USA* **76**:4350–4354 (1979).
21. Morgan, E. T., M. Rönnholm, and J.-Å. Gustafsson. Preparation and characterization of monoclonal antibodies recognizing unique epitopes on sexually differentiated rat liver cytochrome P-450 isozymes. *Biochemistry* **26**:4193–4200 (1987).
22. Kafatos, F. C., C. W. Jones, and A. Efstratiadis. Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. *Nucleic Acids Res.* **7**:1541–1552 (1979).
23. Schreiber, G., A. R. Aldred, T. Thomas, H. E. Birch, P. W. Dickson, G.-F. Tu, P. C. Heinrick, W. Northemann, G. J. Howlett, F. A. de Jong, and A. Mitchell. Levels of messenger ribonucleic acids for plasma proteins in rat liver during acute experimental inflammation. *Inflammation* **1**:59–66 (1986).
24. MacGeoch, C., E. T. Morgan, B. Cordell, and J.-Å. Gustafsson. Growth hormone regulates expression of rat liver cytochrome $P\text{-}450_{16\beta}$ at a pretranslational level. *Biochem. Biophys. Res. Commun.* **43**:782–788 (1987).
25. Cleveland, D. W., M. A. Lopata, R. J. MacDonald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner. Number and evolutionary conservation of α- and β-tubulin and cytoplasmic β- and γ-actin genes using specific cloned cDNA probes. *Cell* **20**:95–105 (1980).
26. Sambrook, J., E. F. Fritsch, and T. Maniatis. *Molecular Cloning, a Laboratory Manual*, Ed. 2, Vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989).
27. Wright, K., and E. T. Morgan. Transcriptional and post-transcriptional suppression of P450IIC11 and P450IIC12 by inflammation. *FEBS Lett.* **271**:59–61 (1990).
28. Birch, H. E., and G. Schreiber. Transcriptional regulation of plasma protein synthesis during inflammation. *J. Biol. Chem.* **261**:8077–8080 (1986).

[2] E. T. Morgan, unpublished observation.
[3] E. T. Morgan and K. Wright, unpublished observations.

29. Marzluff, W. F., and R. C. C. Huang. Transcription of RNA in isolated nuclei, in *Transcription and Translation, a Practical Approach* (B. D. Hames and S. J. Higgins, eds). IRL Press, Oxford, UK, 89–129 (1984).
30. Mode, A., E. Wiersma-Larsson, and J.-Å. Gustafsson. Transcriptional and posttranscriptional regulation of sexually differentiated rat liver cytochrome P-450 by growth hormone. *Mol. Endocrinol.* **3**:1142–1147 (1989).
31. Kasting, N. W., and J. B. Martin. Altered release of growth hormone and thyrotropin induced by endotoxin in the rat. *Am. J. Physiol.* **243**:E332–E337 (1982).
32. Spangelo, B. L., A. M. Judd, P. C. Isakson, and R. M. Macleod. Interleukin-6 stimulates anterior pituitary hormone release *in vitro*. *Endocrinology* **125**:575–577 (1989).
33. Bernton, E. W., J. E. Beach, J. W. Holaday, R. C. Smallridge, and H. G. Fein. Release of multiple hormones by a direct action of interleukin-1 on pituitary cells. *Science (Washington D. C.)* **238**:519–521 (1987).
34. Wehrenberg, W. B., B. A. Janowski, A. W. Piering, F. Culler, and K. L. Jones. Glucocorticoids: potent inhibitors and stimulators of growth hormone secretion. *Endocrinology* **126**:3200–3203 (1990).
35. Armendariz-Borunda, J., J. M. Seyer, A. H. Kang, and R. Raghow. Regulation of TGFβ gene expression in rat liver intoxicated with carbon tetrachloride. *FASEB J.* **4**:215–221 (1990).

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