Transcriptional and post-transcriptional suppression of P450IIC11 and P450IIC12 by inflammation

K. Wright[1,2] and E.T. Morgan[1]

[1]*Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322* and [2]*Department of Biology, Georgia State University, Atlanta, GA 30303, USA*

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Induction of inflammation in rats by treatment with endotoxin or turpentine is known to suppress levels of hepatic mRNAs for P450IIC11 and P450IIC12. We report that transcription of *CYP2C12* in female rats is not significantly reduced from control levels; suppression of this gene during inflammation appears to be mediated post-transcriptionally. In contrast, transcription of *CYP2C11* in male rats is reduced to 23% and to 5% of control levels by turpentine and by endotoxin, respectively. Sex-specificity of *CYP2C11* expression is also regulated transcriptionally, whereas sex-specificity of *CYP2C12* expression appears to be regulated by a post-transcriptional mechanism.

Inflammation; Cytochrome P450; Gene transcription

1. INTRODUCTION

During the acute phase response to infection or inflammation, the synthesis of hepatic 'positive acute phase proteins' (such as α_2-macroglobulin and transferrin) is increased, while the synthesis of 'negative acute phase proteins' (such as albumin and α_{2u}-globulin) is decreased (reviewed in [1]). Many of these changes are manifested at the level of gene transcription [2]. We have recently demonstrated that the synthesis of P450IIC11 and P450IIC12 is also suppressed during an acute phase reaction evoked by treatment with endotoxin or turpentine [3]. These proteins are constitutive, sex-specific hepatic cytochromes P450 in male and female rats, respectively. The present experiments were designed to determine whether this suppression could be attributed to changes in the transcription rates of the corresponding cytochrome P450 genes.

2. MATERIALS AND METHODS

2.1. *Animals*

All procedures were approved by the institutional Animal Care and Use Committee. Sprague-Dawley rats, 6–7 weeks old, were used. Chromatographically pure *E. coli* lipopolysaccharide, serotype 0127:B8 (endotoxin, Sigma Chemical Co., St. Louis, MO) was dissolved in 10 mM sodium phosphate buffer, pH 7.4, 0.15 M NaCl by sonication; animals were given 1 mg/kg i.p. or vehicle. Turpentine (National Solvent Corp., Medina, OH), 5 ml/kg, was injected s.c. at two sites. Animals were killed 24 h after treatment.

Correspondence address: E.T. Morgan, Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322, USA

2.2. *RNA preparation and slot blot analysis*

Total hepatic RNA was prepared, and the levels of P450IIC11, P450IIC12, and β-actin mRNAs were determined by slot blot using cloned cDNAs as previously described [3]. Poly(A^+) RNA was isolated by batch absorption using oligo(dT)-cellulose and used in some experiments for slot blot analysis. The sources of cDNA probes used in this study were reported previously [3].

2.3. *Transcription assays*

Hepatic nuclei were isolated as described by Blobel and Potter [4]. Transcription assays were performed using methods modified from Birch and Schreiber [2] and Marzluff and Huang [5]. Nuclei (aprox. 200 μg DNA) were incubated in a total volume of 200 μl 50 mM Hepes, pH 7.5, containing 50 mM KCl, 2.5 mM $MgCl_2$, 50 μM EDTA, 5 mM dithiothreitol, 25% glycerol, 250 μM each of ATP, GTP, and CTP, 2 mM creatinine phosphate, 2 μg creatine kinase, 7 units RNase inhibitor (5 Prime – 3 Prime, Inc., West Chester, PA), and 100 μCi [α-^{32}P]UTP. After 30 min at 25°C, 89 units RNase-free DNaseI were added and the incubation was continued for 30 min. Proteinase K (40 μg) and vanadyl ribonucleoside complex (at a final concentration of 2 mM) were added, and the samples were incubated at 37°C for 30 min. Nuclei were lysed by the addition of 10 volumes of 10 mM EDTA, 1% SDS, pH 8.0. The solution was extracted with hot phenol/chloroform/isoamyl alcohol (50:24:1), and the RNA was recovered by ethanol precipitation.

Linearized pBR322 plasmids containing P450IIC11 (5 μg), P450IIC12 (10 μg), and β-actin (5 μg) cDNA were denatured and applied to a Nytran filter (Schleicher and Schuell, Keene, NH) using a slot-blot apparatus [5]. The DNA was immobilized by UV irradiation and the filters were prehybridized overnight at 42°C in hybridization buffer (50% formamide, 5×SSPE, 5× Denhardt's solution, 1% SDS, 0.1 mg/ml salmon sperm DNA and 0.9 mg/ml poly(A)). The [^{32}P]RNA was hybridized to the immobilized DNA at a concentration of approx. 4×10^6 cpm/ml for 48 h at 42°C. Blots were washed twice for 30 min at 22°C in 2× SSC, 0.1% SDS and three times for 30 min at 60°C in 0.1×SSC, 0.1% SDS. The intensities of the autoradiographic bands were quantified by densitometric scanning using an LKB Ultroscan XL laser densitometer. Results were corrected for the amount of input labelled RNA.

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3. RESULTS AND DISCUSSION

3.1. *Transcription assay validation*

Nuclear RNA synthesis was linear with respect to the volume of added nuclei, α-amanitin-sensitive (2 μg/ml) RNA synthesis was linear with time to 40 min. There was no detectable hybridization of the labelled RNA to 5 μg immobilized pBR322 (Fig. 1). Labelled RNA synthesized by nuclei from male rats in the presence of 2 μg/ml α-amanitin did not hybridize to the P450IIC11 probe (not shown). Under our conditions, the densitometric signals were proportional to the amount of input RNA for all probes.

3.2. *Sex-specificity of P450 gene transcription*

RNA synthesized by nuclei isolated from female rat livers did not hybridize to the P450IIC11 probe (Fig. 1). This result confirms the specificity of our hybridization procedures for *CYP2C11* transcription. Previous work has suggested that the male-specific expression of *CYP2C11* is partially mediated at the post-transcriptional level [6]. However, our data suggest that transcription alone is sufficient to account for the sex-specific expression of P450IIC11. In contrast, labelled RNA synthesized in nuclei isolated from male rat livers hybridized to the P450IIC12 probe; this hybridization was 49% of that observed with RNA synthesized in nuclei isolated from female rat livers (Fig. 1). The apparent lack of sex-specificity for *CYP2C12* transcription is similar to that seen by Mode et al. [6], although hepatic P450IIC12 mRNA in our males, measured with the same probe, was 3% of that in the females (not shown). Thus, although the probe for P450IIC12 appears to hybridize specifically with its mature mRNA, our data cannot rule out cross-reaction with other nuclear cytochrome P450 mRNAs in our procedure. Alternatively, these data may reflect a post-transcriptional mechanism for P450IIC12 regulation in female rats.

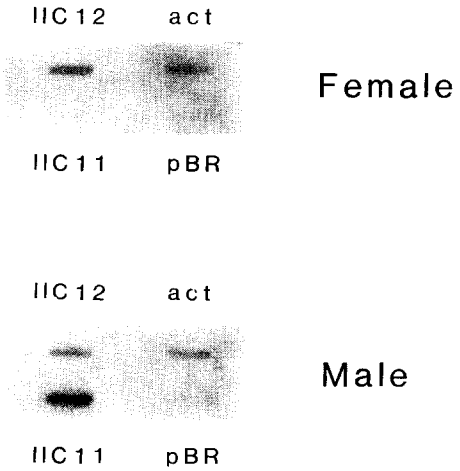

Fig. 1. Specificity of hybridization of nuclear [^{32}P]RNA to immobilized DNA probes (act = β-actin).

Table I

Effect of inflammation on *CYP2C11* and *CYP2C12* gene transcription rates in rat liver nuclei

Sex/treatment (n)	Gene transcription rate (% of controls)		
	CYP2C11	actin	CYP2C12
Male			
Control (3)	100 ± 30	100 ± 49	
Endotoxin (3)	5 ± 5*	98 ± 49	
Turpentine (3)	23 ± 20*	143 ± 41	
Female			
Control (4)		100 ± 41	100 ± 31
Turpentine (3)		62 ± 20	61 ± 21

*$P<0.05$, significantly different from control group

3.3. *Inflammation*

Treatment of female rats ($n=3$) with turpentine reduced *CYP2C12* transcription to 61% of the control levels (Table I) and β-actin transcription to 62% of the controls; however, individual variation was high, and these results were not statistically significant. The level of P450IIC12 mRNA in total hepatic RNA from these rats was reduced to 36% of controls ($P<0.05$, not shown); β-actin mRNA was not affected by treatment. Therefore, subject to the above caveat regarding the specificity of the P450IIC12 probe, our data do not support the hypothesis that a change in transcription rate accounts for the suppression of mRNA for P450IIC12 seen in this model. Rather, they suggest that this change is more likely due to post-transcriptional mechanisms.

Turpentine-induced inflammation suppressed transcription of *CYP2C11* in nuclei of male rats to 23% of controls (Fig. 2 and Table I), whereas there was no significant effect on transcription of the β-actin gene. Treatment of the rats with endotoxin had an even greater effect on *CYP2C11* transcription (5% of controls, Fig. 2 and Table I) without affecting β-actin. Slot blot assays on hepatic poly(A$^+$) RNA from these rats (not shown) indicated that P450IIC11 mRNA was suppressed to 10% and 20% of control male levels ($P<0.05$) by turpentine and endotoxin, respectively, confirming our previous observations [3]. Since the magnitudes of the decreases in *CYP2C11* gene transcription are similar to or greater than those of the

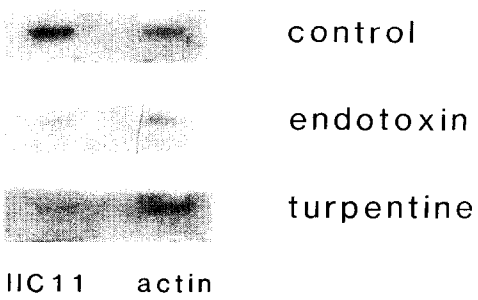

Fig. 2. Effects of inflammation on hybridization of nuclear [^{32}P]RNA to immobilized P450IIC11 and β-actin probes.

P450IIC11 mRNA, our data suggest that inflammation regulates expression of *CYP2C11* by reducing the transcription rate of the gene. Thus, there may also be a sex difference in the effects of inflammation on the expression of these cytochromes P450.

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