Down-Regulation of the Expression of Three Major Rat Liver Cytochrome P450S by Endotoxin In Vivo Occurs Independently of Nitric Oxide Production

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ABSTRACT

Endotoxemia results in both the down-regulation of multiple cytochrome P450 genes and the induction of inducible nitric oxide synthase (NOS2). The nitric oxide (NO) released during inflammation has been implicated as the mediator of the decreased catalytic activity and expression of several cytochrome P450 isozymes. We examined the role of NO in the decreases of both gene expression and activity of three major P450s in the endotoxic Fischer 344 rat. Endotoxin (LPS) treatment suppressed both mRNA and protein expression of P450 2C11, 2E1, and 3A2. Coadministration of the NOS inhibitor aminoguanidine to LPS-treated rats completely inhibited the release of NO into the plasma but did not reverse the down-regulation of expression of any of the P450s examined at three time points. LPS treatment had a biphasic effect on some P450 catalytic activities. The hydroxylation of testosterone at the 2α-, 16α- and to a lesser extent 6β-positions, was inhibited 6 hr after LPS treatment and returned to normal by 12 hr. The role of NO in the 6 hr effects could not be assessed due to effects of the aminoguanidine treatment itself. The second phase of decreased P450 activities seen after 24 hr was attributed to the NO-independent decrease in gene expression. Our results suggest that NO is not required for the LPS-evoked down-regulation of P450 2C11, 2E1 and 3A2 mRNA or protein expression. We cannot rule out a possible role for NO in the decreases in P450 activities seen early in the response.

The cytochrome P450 superfamily is a group of enzymes that catalyze the oxidation and reduction of a wide variety of chemical structures. P450 enzymes expressed in the liver are responsible for the bioactivation and/or detoxification of drugs and toxic chemicals (Porter and Coon, 1991). Stimulation of the immune system during infection or inflammation results in an impairment of P450 metabolic activity and a decrease in the total hepatic P450 content (Morgan, 1997). Many P450s are suppressed at the level of mRNA expression, and the expression of P-450 2C11 is suppressed at the transcriptional level following treatment with bacterial LPS or turpentine (Morgan, 1997). In vivo and in vitro studies have shown that the cytokines IL-1, IL-6 and TNF-α as well as IFN or IFN inducers can mimic the down-regulation of P450 gene products seen during infection or inflammation (Chen et al., 1995; Abdel-Razzak et al., 1993; Knickle et al., 1992; Morgan et al., 1994).

Cytokines also induce NOS2 and result in the production of NO in certain cells including hepatocytes, macrophages, endothelial cells and leukocytes during a cellular immune response (Nathan, 1992). NO affects the activities of a number of enzymes, due to its ability to bind to heme and nonheme iron complexes (Ignarro et al., 1986; Kanner et al., 1992; Wink et al., 1993). Various studies involving treatment of whole animals with LPS, or incubation of rat or human hepatocytes with LPS, cytokines and/or interferons, have implicated NO as a mediator of the decreases seen in P450 activities. Treating microsomes with NO-generating or NO has also been shown to inhibit P450 activity (Wink et al., 1993; Khatserenko et al., 1990, 2B1/2, 2B6 and 3A4 (Donato et al., 1990) metabolic activities. The reversible phase of inhibition is due to the formation of an iron-nitrosyl complex with the ferrous P450 heme (Wink et al., 1993; Minamiyama et al., 1997). A second, irreversible inhibitory phase was attributed to oxidation of critical amino acids in P450, resulting in loss of catalytic activity (Wink et al., 1993). Supporting this notion, high concentrations of NO

ABBREVIATIONS: LPS, bacterial endotoxin; IL, interleukin; TNF, tumor necrosis factor; IFN, interferons; NOS2, inducible nitric oxide synthase; NO, nitric oxide; GAP, glyceraldehyde-3-phosphate dehydrogenase; AG, aminoguanidine; NOx, nitrate plus nitrite; L-NAME, Nω-nitro-L-arginine methyl ester.

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result in the in vitro nitrination of a tyrosine near the active site of CYP2B4 (Quaroni et al., 1996). NO-mediated loss of P450 heme in isolated hepatocytes has also been reported (Kim et al., 1995).

It has been reported that NO is also the mediator of the decreases seen in P450 2C11, 3A2, 2B1/2 and 1A2 protein expression after incubation of cytokines with primary hepatocytes (Carlson and Billings, 1996). These findings are supported by the report by Khatsenko and Kikkawa (1997) that NOS inhibitors are capable of reversing the decreases in P450 2C11, 3A2, 2B1/2 and 1A2 activity, protein and mRNA expression in rats treated with LPS. However, our laboratory has demonstrated NO-independent cytokine- or LPS-mediated down-regulation of P450 2C11 mRNA and protein expression (Sewer and Morgan, 1997) in cultured rat hepatocytes. Monshouwer et al. (1996) similarly reported no effect of NOS inhibition on cytokine-evoked decreases in P450-catalyzed steroid hydroxylase activities in porcine hepatocytes.

To attempt to resolve the discrepancies between our in vitro work and the results of other laboratories, we studied the effects of NOS inhibition on the down-regulation of three major rat P450 enzymes in LPS-treated rats. We report an NO-independent suppression of P450 2C11, 3A2 and 2E1 in rats treated with LPS. These findings support our previous studies in cultured hepatocytes showing no role of NO in the LPS or IL-1β-evoked down-regulation of P450 2C11 (Sewer and Morgan, 1997) and our in vivo studies demonstrating that irritants are capable of down-regulating P-450 2C11 without inducing NOS2 (Sewer et al., 1997). Our results do not exclude a role for NO in the decreases in P450 activities seen early in the response to LPS.

**Materials and Methods**

**Animals and treatments.** Male Fischer 344 (Harlan Sprague-Dawley, Indianapolis, IN) 6 to 8 wk old were used. The animals were allowed free access to food and water at all times. Chromatographically purified Escherichia coli LPS, serotype 0127:B8 (Sigma Chemical Co., St. Louis, MO), was dissolved in sterile 0.9% saline and injected i.p. at a dose of 1 mg/kg body weight. Control animals received an equivalent volume of sterile saline. AG (133 mg/kg, i.p.) was administered to rats beginning 30 min after a single injection of LPS or saline treatment, and every 4 hr thereafter. The animals were killed by CO2 asphyxiation at 2, 4, 6, 12 and 24 hr after injection of LPS. These procedures were approved by the Institutional Animal Care and Use Committee of Emory University.

**Analysis of plasma nitrite and nitrate concentration.** The stable end products of L-arginine-dependent NO synthesis, nitrate and nitrite, were measured in the plasma using a colorimetric method based on the Griess reaction (Tracey et al., 1995; Grisham et al., 1996). Briefly, aliquots of plasma were added to 35% sulfosalicylic acid and vortexed every 5 min for 30 min to deproteinize the samples. The samples were then centrifuged at 10,000 × g at 4°C for 15 min. An aliquot of the supernatant was taken for nitrite and nitrate analysis. Twenty microliters of plasma sample were mixed with 20 ml of 0.31M phosphate buffer (pH 7.5), 10 ml of 0.1 M flavin adenine dinucleotide (FAD), 10 ml of 1 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 10 ml of nitrate reductase (10 U/ml) and 30 ml H2O in a 96-well plate. The reaction was allowed to proceed for 1 hr in the dark. The efficiency of conversion 2oxygen of nitrate to nitrite was 98%. To each sample, 1 µl of lactate dehydrogenase (1500 U/ml) and 10 µl of 100 mM pyruvic acid were added, and samples were incubated for 15 min at 37°C. The samples were then mixed with an equivalent volume of Griess reagent [1:1 mixture of 1% sulfanilamide in 0.05% H3PO4 and 0.1% N-(1-naphthyl)ethylenediamine] and incubated for an additional 10 min at room temperature. Nitrite levels were determined colorimetrically at 550 nm with a Thermomax microplate reader (Molecular Devices, Sunnyvale, CA) and a sodium nitrite standard curve.

**Preparation of microsomes and total RNA.** Livers were excised and perfused with cold 1.15% KCl. Pyrophosphate-washed microsomes were prepared as described by Haugen and Coon (1976). Total RNA was prepared according to the method of Chomczynski and Sacchi (1987). Purified microsomes and total RNA were stored at −80°C.

**RNA Northern and slot blots.** In samples of total hepatic RNA, the RNA concentration was determined spectrophotometrically at 260 nm. Northern blotting was performed as described by Sambrook et al. (1989). In short, formaldehyde-containing agarose gels (1%) were used to subject denatured RNA to electrophoresis at 70 V for 4 hr. The RNA was blotted onto MagnaGraph nylon transfer membrane filters (Micron Separations Inc. Westboro, MA) overnight and was fixed by both UV irradiation and baking at 80°C. The blots were hybridized to cDNA or oligonucleotide probes, washed and subjected to autoradiography. A cDNA probe for GAP was used to control for loading and transfer artifacts.

Slot blots were prepared as described previously (Morgan et al., 1994). Total RNA was denatured using formaldehyde, and 2 µg of RNA for each sample were loaded onto Nitran maximum strength filters (Schleicher & Schuell, Keene, NH) in the wells of the slot blot apparatus. The RNA was immobilized by both UV irradiation and baking at 80°C. The filters were hybridized, probed, washed and exposed to film in the same manner as Northern blots. These conditions were previously determined to give linearity of the signals with the amount of RNA applied. Quantitative analysis of autoradiographs was done by video (Lynx Densitometer, Applied Imaging Corp., Santa Clara, CA) or laser (Personal Densitometer, Molecular Dynamics Ltd., Sunnyvale, CA). The relative content of poly (A)+ RNA in the samples, measured by probing with 32P-labeled oligo-d(T), was used to normalize slot blot data (Morgan et al., 1994). **cDNA and oligonucleotide probes.** Relative levels of CYP2C11, CYP2E1 and β-fibrinogen mRNAs were quantitated by Northern and slot blot assays using full length cDNAs for CYP2C11 and CYP2E1, as described previously (Sewer et al., 1997). CYP3A2 mRNA was detected using an oligonucleotide complementary to nucleotides 1690–1729 (Gonzalez et al., 1986). The Megaprime labeling kit (Amersham Corp., Arlington Heights, IL) and [γ-32P]ATP were used to radiolabel cDNA probes. T4 polynucleotide kinase and [γ-32P]ATP were used to 5′-end radiolabel oligonucleotide probes. All blots probed with cDNA probes were hybridized at 42°C and washed at 62°C. The hybridization and stringency wash conditions for the oligonucleotide probes have been described before (Morgan et al., 1994). Bound 32P-labeled probes were detected by autoradiography and quantified by analysis on either a Lynx video densitometer (Applied Imaging, Santa Clara, CA) or a Personal laser densitometer (Molecular Dynamics Ltd., Sunnyvale, CA). All assays were performed under previously established conditions of linearity between the amount of the target mRNA on the filter and the densitometric response.

**Assays of hepatic microsomes.** Total microsomal protein was determined by the method of Lowry et al. (1951). Cytochrome P450 concentrations were determined from the CO difference spectrum of the reduced protein at 450 nm (Omur and Sato, 1964).

**Western blot immunassays.** The relative levels of various P450 isoforms in the microsomes were measured by Western blotting. Proteins were separated by polyacrylamide gel electrophoresis (7.5% polyacrylamide) in the presence of sodium dodecyl sulfate, and electrophoretically blotted onto nitrocellulose membranes (Schleicher & Schuell). Procedures for measuring P-450 2C11, 2B1 and 3A2 have been described previously (Morgan et al., 1994). The anti-

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bodies to P450 2E1 and 3A2 were generous gifts from Dr. Magnus Ingelman-Sundberg, Karolinska Institute (Stockholm, Sweden) and Dr. James Halpert (University of Arizona), respectively. The binding of all antibodies were detected using the ECL detection system (Amersham Life Sciences, Arlington Heights, IL) according to the manufacturer's instructions. The intensities of the stained bands were measured by laser densitometry, and were determined to be proportional to the amount of antigen loaded on the blot within the experimental range used.

**Microsomal P450 activities.** The specific activities of P450 2C11 and 3A2 were determined by measurement of 2α and 16α- (2C11) and 6β-testosterone hydroxylation (3A2) (Ciaccio and Halpert, 1989; Waxman, 1991). Briefly, 50 μg microsomal protein were preincubated for 5 min at 37°C in a buffered solution containing 250 μM [4-14C]testosterone. The reaction was started with the addition of 1 mM NADPH and ascorbic acid; 10 mM NADPH was added to initiate the reaction. The reaction was allowed to proceed for 10 min. The reaction was stopped by the addition of tetrahydrofuran, and aliquots were spotted on the preabsorbent loading zone of a silica gel TLC plate [250 μM, Si250F (19C)]. The plates were developed twice in dichloromethane:acetone (4:1, v/v), and the radioactive areas on the plates were scraped and quantified by liquid scintillation counting. Metabolites were localized by autoradiography and identified by comparison with unlabeled standards (Steraloids, Inc., Wilton, NH). The p-nitrophenol hydroxylation activity of P450 2E1 was assayed spectrophotometrically (Koop, 1986). A total of 300 μg of microsomal protein was preincubated in a phosphate buffer containing 0.2 mM p-nitrophenol and ascorbic acid; 10 mM NADPH was added to initiate the reaction. The reaction was allowed to proceed for 10 min and was then stopped by the addition of 1.5 N perchloric acid and placed on ice for 10 min. After a 10-min spin at 4000 rpm at 4°C, the supernatant was mixed with 10 N NaOH and the absorbance read at 510 nm. Control assays were performed to ensure linearity of both time and protein concentration.

**Statistical analysis.** Data from slot-blot and Western blot assays were expressed as the percentage of the mean of the control group in the presence and absence of the competitive NOS inhibitor AG at time points ranging from 6 to 24 hr. Figure 2 shows a Northern blot analysis of the total RNA prepared from rats treated for 24 hr. The expression of P450 2C11 and 3A2 were suppressed by AG, and remained suppressed in animals treated with both AG and LPS (fig. 2). Figure 3 shows graphs generated from slot blots of RNA isolated from animals treated for 6, 12 and 24 hr with LPS ± AG. Levels of P450 2C11 mRNA were significantly reduced 6 hr after injection, and reached 9% of control after 24 hr (fig. 3A). The concomittant of NO detected in the plasma of rats after treatment with AG. An increase in plasma NOx was first detected at 4 hr after LPS treatment, and the maximal increase to 518 μM NOx occurred at 6 hr. After 12 hr of LPS treatment, plasma NO levels remained increased 9-fold over the saline-treated control animals. The administration of repeated doses of 133 mg/kg AG every 4 hr to LPS-treated rats significantly reduced the release of NO into the plasma at the 4, 6 and 12 hr time points (fig. 1). In a similar experiment, a lower dose of AG (50 mg/kg) was administered to LPS-treated rats for 6 hr. This lower dose was not effective in returning plasma nitrite and nitrate levels back to levels seen in saline treated rats. Twenty-four hr of LPS treatment did not result in a significant increase in plasma NO levels. AG when administered alone had no significant effect on plasma NO concentrations (fig. 1).

**AG and LPS-evoked suppression of P450 mRNAs.** Total RNA was isolated from rats treated with LPS in the presence and absence of the competitive NOS inhibitor AG at time points ranging from 6 to 24 hr. Figure 2 shows a Northern blot analysis of the total RNA prepared from rats treated for 24 hr. The expression of P450 2C11 and 3A2 were suppressed by AG, and remained suppressed in animals treated with both AG and LPS (fig. 2). Figure 3 shows graphs generated from slot blots of RNA isolated from animals treated for 6, 12 and 24 hr with LPS ± AG. Levels of P450 2C11 mRNA were significantly reduced 6 hr after injection, and reached 9% of control after 24 hr (fig. 3A). The concomittant of NO detected in the plasma of rats after treatment with LPS and/or AG. An increase in plasma NOx was first detected at 4 hr after LPS treatment, and the maximal increase to 518 μM NOx occurred at 6 hr. After 12 hr of LPS treatment, plasma NO levels remained increased 9-fold over the saline-treated control animals. The administration of repeated doses of 133 mg/kg AG every 4 hr to LPS-treated rats significantly reduced the release of NO into the plasma at the 4, 6 and 12 hr time points (fig. 1). In a similar experiment, a lower dose of AG (50 mg/kg) was administered to LPS-treated rats for 6 hr. This lower dose was not effective in returning plasma nitrite and nitrate levels back to levels seen in saline treated rats. Twenty-four hr of LPS treatment did not result in a significant increase in plasma NO levels. AG when administered alone had no significant effect on plasma NO concentrations (fig. 1).

**Results**

**Products of NO in plasma.** Figure 1 shows the concen-

![Fig. 1. Plasma NO levels after treatment of rats with LPS ± AG. F344 rats were injected i.p. with 1 mg/kg LPS followed by repeated doses of 133 mg/kg AG at 4-hr intervals. Rats were killed at 2, 4, 6, 12 and 24 hr after treatment. Plasma concentrations of nitrite plus nitrate were analyzed via the Griess reaction as described in "Materials and Methods." The data presented represent the means ± S.E.M. of five rats per treatment group and expressed as the concentration (μM) of nitrite + nitrate released. *Significantly different from saline treated rats, P < .05.**

![Fig. 2. Northern blot of P450 mRNA expression in endotoxemic rats treated with AG. Total RNA was isolated from rats treated for 24 hr with LPS in the presence and absence of AG, and subjected to Northern blot analysis as described in "Materials and Methods." The autoradiograms show represent three samples from each treatment group, taken from nonadjacent lanes in the same blot. Nominally, 10 μg of total RNA were loaded in each lane. The blot was probed sequentially for expression of the different P450s, and for GAP to ensure equal loading.](http://www.jpet.aspetjournals.org/article/354/4/354/S1)
tion of AG to the animals had no effect on the LPS-evoked suppression of P450 2C11, 2E1 and 3A2 mRNAs. Rats were treated as described in “Materials and Methods.” Total RNA was subjected to slot-blot analysis and the blots probed for 2C11 (A), 2E1 (B) and 3A2 (C) expression. All slot-blots were normalized to the poly(A⁺) content using a blot probed with oligo-dT₃₀. The data represent the means ± S.E.M. of five rats per treatment group, and results are expressed as a percentage of the saline-treated group mean for each time point. *Significantly different from saline treated rats, P < .05.

The effect of AG on LPS-mediated down-regulation of P450 protein levels. Figure 4 shows Western blots of P450 2C11, 3A2 and 2E1 expression following treatment with LPS and/or AG for 24 hr. As depicted graphically in figure 5, LPS down-regulated P450 2C11 and 3A2 at the 24 hr time point to 35 and 46% of control, respectively (figs. 5A and C). The decreases in P450 protein levels were not significantly affected by AG treatment. P450 2E1 protein was not significantly affected by LPS throughout the entire time course. However, AG did significantly induce P450 2E1 expression at the 6- and 12-hr time points (fig. 5B). Total microsomal P450 content was significantly decreased to 77 and 76%, respectively, after 12 and 24 hr of LPS treatment (fig. 5D). Coadministration of AG was unable to attenuate the LPS-evoked decreases in P450 content. Although LPS alone did not affect total P450 content at the 6-hr time point, AG treatment significantly decreased total P450 content when administered alone (75% of control) and to LPS-treated rats (65% of control). Twelve hours of exposure to LPS decreased total P450 content, and coadministration of LPS and AG for 24 hr caused a further significant decrease in P450 content despite the lack of effect of AG alone.

P450-catalyzed activities. The graphs in figure 6 show the effects of LPS, AG or both, on hepatic microsomal testosterone hydroxylase activities. P450 2C11, 2B and 2C11, and 3A2 catalyze the hydroxylation of testosterone at the 2α, 16α and 6β positions, respectively (Waxman, 1988). We were unable to detect any significant formation of the 16β metabolite, reflecting the very low constitutive expression of P450 2B isoforms (Waxman, 1988).
The various treatments produced very similar effects on testosterone 2α- and 16α-hydroxylase activities (fig. 6A,B), reflecting the fact that both are mainly catalyzed by P450 2C11 in uninduced male rats (Waxman, 1988). In LPS-treated rats, both activities showed a transient decrease of about 20 to 30% 6 hr after treatment (fig. 6); no effect at 12 hr; and, a second decrease of 30 to 45% at 24 hr. AG treatment alone also caused decreases in these P450 2C11-dependent activities at the 6- and 24-hr time points (fig. 6), so it was not possible to determine if NOS inhibition could reverse the effect of LPS. If anything, the effects of AG and LPS appeared to be additive.

The time course of the LPS effect on P450 3A2-catalyzed testosterone 6β-hydroxylase activity was similar to that of the P450 2C11-catalyzed activities, except that the decrease at 6 hr was smaller and not statistically significant (fig. 6C). No significant effect of AG alone on this activity was detected. At 24 hr after treatment, the activity of the LPS + AG group was not significantly different from control. It also was not significantly different from the group treated with LPS alone. Therefore, it is not possible to conclude whether AG could partially reverse the LPS effect, or not.

Hydroxylation of p-nitrophenol, an index of P450 2E1 metabolic activity, was also examined. No effect of LPS alone on this activity was observed until 24 hr after treatment, when it was decreased to 41% of control (fig. 7). AG treatment alone significantly decreased the p-nitrophenol hydroxylase activity at the 6-hr time point only (fig. 7). AG exposure had no effect on the LPS-evoked decrease in p-nitrophenol hydroxylase activity at the 24-hr time point.

**Discussion**

The down-regulation of hepatic P450s of the 1A, 2B, 2C, 2E and 3A subfamilies after administration of agents that stimulate an inflammatory response is well established. It is also known that the NO released after treatment with agents that stimulate an immune response is capable of binding to the iron containing prosthetic groups of several enzymes. We have demonstrated previously in cultured primary rat hepatocytes that the LPS or IL-1β-stimulated down-regulation of P450 2C11 mRNA and protein expression occurs via an NO-independent pathway. In this report, we find that the down-regulation of P450 2C11, 3A2 and 2E1 mRNA and protein expression in the in vivo LPS model of inflammation is also independent of NO generation. We found that administration of AG at a dose of 133 mg/kg in 4-hr intervals proved to be the most effective method for inhibiting release of NO. In preliminary studies (not shown) we found that injecting a single dose of AG was not effective at attenuating release of NO into the plasma of LPS-treated rats.

LPS was able to decrease the mRNA and protein expression of all three P450 isoforms examined both in the presence and absence of doses of AG which completely inhibited NO release. The findings in our study are in agreement with our recent in vitro study demonstrating NO-independent down-regulation of P450 2C11 expression after treating hepatocytes with either IL-1β or LPS (Sewer and Morgan, 1997). This study also supports our in vivo results in rats treated with particulate irritants, which resulted in the down-regulation of P-450 2C11 but no induction of NOS2 (Sewer et al., 1997).

In contrast, in a recent study Khatsenko and Kikkawa (1997) reported that AG and another NOS inhibitor L-NAME were capable of reversing the down-regulation of constitutively expressed P450 2C11, 3A2, 1A2 and 2B1/2 in endotoxemic rats. At the present time it is unclear why our results differ from those of Khatsenko and Kikkawa (1997). The efficiencies of inhibition of NO production in our study and theirs were similar. In their work, LPS exposure resulted in a significant increase in plasma nitrite and nitrate concent-
trations 24 hr after administration. This is at odds with our study, in which NOx levels were elevated at 6 and 12 hr after LPS treatment, and declined to basal levels by 24 hr. In the study by Khatsenko and Kikkawa (1997), they presented data using mainly L-NAME as the NOS inhibitor, and studied a single time point (24 hr). The differences between their observations and ours may be due in part to effects of the respective inhibitors employed (L-NAME and AG) that may be unrelated to NOS inhibition, and also to the fact that the magnitudes of suppression of P450 2C11 and 3A2 mRNA that they achieved after 24 hr of LPS treatment were considerably less than in our study. Although Khatsenko and Kikkawa (1997) treated animals with AG and saw partial reversal of the decreases in P450 1A1/1A2-dependent activity and CYP2B1/2 protein, they did not report its effects on down-regulation of P450 2C11 or 3A2 expression or the activities of these enzymes. The effects of AG treatment alone were also not reported.

It is possible that strain differences (Fischer 344 rats in our study vs. Sprague-Dawley rats in the Kikkawa study) may account for the dissimilarities between the two studies. We have previously found differences, both in the induction of the P450 4A subfamily and in variability in the suppression of P450 2C11, in Sprague-Dawley vs. Fischer 344 rats after LPS treatment (Sewer et al., 1995). However, we believe that it is more likely that nonspecific effects of one or both of the NOS inhibitors used in the two studies are confounding clear interpretation of the data. Mice with selective inactivation of the Nos2 gene are now available, and afford the opportunity to study the role of NOS in this phenomenon without the use of drugs. This avenue is currently being pursued in our laboratory.

The biphasic effect of LPS treatment on the catalytic activity of P450 2C11 is striking. It seems clear that the decreases in testosterone 2α- and 16α-hydroxylase activities at 24 hr are due to the NO-independent decrease in P450 2C11 gene expression, because there is no detectable NO production at 24 hr. However, the decrease at 6 hr occurs in the absence of a detectable decrease in P450 2C11 protein or in total microsomal P450 content. A similar pattern is seen for testosterone 6β-hydroxylase and P450 3A2, although the decrease in activity at 6 hr was not significant. One could speculate that the effects of LPS at 6 hr on some P450-dependent catalytic activities reflect a reversible inhibition of the enzyme by NO, because the transient inhibition of P450 catalytic activities that we observe after LPS treatment is no longer apparent by 12 hr. Based on our data, it also appears that P450 isoforms may be differentially susceptible to this NO inhibitor component. However, it seems unlikely that a reversible P450 heme-nitrosyl complex (Wink et al., 1993) would be stable under the conditions of microsomal preparation, which includes a pyrophosphate wash step, because the microsomal P450-NO complex (detected by electron spin resonance) rapidly dissociates (Minamiyama et al., 1997). Because apo-P450 formed by incubation with NO in hepatocytes can be reconstituted with added heme (Kim et al., 1995), it is formally possible that the decrease reflects a reversible NO-evoked loss of P450 heme. The possibility should also be considered that NO formed in vivo could affect P450 catalytic activity indirectly, by regulating the formation of some other modulatory factor in hepatocytes or nonparenchymal cells. Because of the observed inhibitory effects of AG on P450 catalytic activities we could not determine the ability of NOS inhibition to prevent the early decreases in P450 activities. AG has been shown to inhibit P450 catalytic activities in microsomal preparations (Clement et al., 1994). The use of L-NAME instead of AG may not alleviate this problem, because Khatsenko and Kikkawa (1997) also saw effects of this agent alone on catalytic activities. Again, it appears that the use of NOS2 knockout mice may provide a more definitive answer to this question.

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