Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 11147–11151, December 1993
Pharmacology

Nitric oxide is a mediator of the decrease in cytochrome P450-dependent metabolism caused by immunostimulants

(cytokines/lipopolysaccharide/hepatic metabolism)

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ABSTRACT **Bacterial lipopolysaccharide (LPS) and a diverse array of other immunostimulants and cytokines suppress the metabolism of endogenous and exogenous substances by reducing activity of the hepatic cytochrome P450 mixed-function oxidase system. Although this effect of immunostimulants was first described almost 40 yr ago, the mechanism is obscure. Immunostimulants are now known to cause NO overproduction by cells via induction of nitric oxide synthase. We have investigated whether NO overproduction is involved in suppressing hepatic metabolism by LPS. *In vitro* treatment of hepatic microsomes with NO, produced by chemical decomposition of 3-morpholinosydnonimine or by nitric oxide synthase, substantially suppressed cytochrome P450-dependent oxygenation reactions. This effect of NO was seen with hepatic microsomes prepared from two species (rat and chicken) and after exposure to chemicals that induce distinct molecular isoforms of cytochromes P450 (β-naphthoflavone, 3-methylcholanthrene, and phenobarbital). Spectral studies indicate that NO reacts *in vitro* with both Fe^{2+}- and Fe^{3+}-hemes in microsomal cytochromes P450. *In vivo*, LPS diminished the phenobarbital-induced dealkylation of 7-pentoxyresorufin by rat liver microsomes and reduced the apparent P450 content as measured by CO binding. These LPS effects were associated with induction of NO synthesis; LPS-induced NO synthesis showed a strong positive correlation with the severity of cytochrome P450 inhibition. The decrease in both hepatic microsomal P450 activity and CO binding caused by LPS was largely prevented by the selective NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester. Our findings implicate NO overproduction as a major factor mediating the suppression of hepatic metabolism by immunostimulants such as LPS.**

NO is a secretory product of mammalian cells that is important in the regulation of vascular tone, platelet function, neurotransmission, and host-defense mechanisms (1–3). These physiological actions are attributable to the oxidation by NO of heme and nonheme iron and iron–sulfur complexes in the active sites of key metabolic enzymes. Thus, through its interaction with iron, NO modulates the activity of target proteins. Although NO is normally produced in relatively small quantities and presumably by particular cell types (e.g., endothelial cells and neurons), NO synthesis increases substantially and appears in numerous cell types (smooth muscle, Kupffer cells, macrophages, and hepatocytes) after exposure to immunological stimuli such as bacterial lipopolysaccharide (LPS) (2, 3).

Cytochromes P450 are a major class of heme-containing proteins, defined by their characteristic absorption maximum at 450 nm upon CO binding. These enzymes are enriched in hepatic microsomes, where they catalyze the oxidative metabolism of endogenous and exogenous substances (for review, see refs. 4–6). Cytochromes P450 comprise a large, but closely related, superfamily of distinct gene products with different substrate specificities. Specific molecular isoforms of P450 are preferentially induced by exposure to chemical stimuli such as phenobarbital (PB) (CYP2B1/2), β-naphthoflavone (β-NF), and 3-methylcholanthrene (3-MC) (CYP1A1/2) (4–7).

Immunological stimuli depress cytochrome P450-mediated hepatic metabolism of a variety of drugs and endogenous substances. Indeed, attenuated cytochrome P450 activity has been seen in animals after infection with bacteria and viruses or after treatment with cytokines (e.g., interleukin 1; interferon γ, tumor necrosis factor α) and immunostimulants (e.g., LPS and single-stranded RNA) (8, 9). Despite wide acceptance that the immune system can inhibit hepatic drug metabolism, the mechanism of this effect is largely unknown. Our study reveals that NO, produced by immunoactivated cells, mediates suppression of cytochrome P450 enzymes *in vitro* and *in vivo*.¶

MATERIALS AND METHODS

Preparation of Hepatic Microsomes. Microsomal cytochromes P450 were induced in livers as follows: Chicken embryos at 16–17 days old were treated with 6.7 mg of β-NF in water by direct injection into the egg 48 hr before removal of the liver for microsome preparation. PB was administered to male Wistar rats (250–350 g) by i.p. injection in saline, at doses of 80 mg/kg, given at 24-hr intervals for 3 consecutive days. 3-MC was similarly administered to rats by i.p. injection of 40 mg/kg in corn oil, at 24-hr intervals for 3 consecutive days. Rats were killed by PB overdose. Microsomes were prepared from liver homogenates as the 100,000 × *g* pellet, and the hepatic microsomal cytochromes P450 and b_5 were quantified spectrophotometrically by a described method (11).

To test the effect of NO production *in vivo* on hepatic cytochromes P450 activity, rats were treated with saline (control) or PB (as described above). In one group, after treatment with PB, LPS was administered as a single injection (2 or 4 mg/kg i.p.) 6 or 15 hr before killing the animals and preparing microsomes. Another group received LPS in combination with N^{ω}-nitro-L-arginine methyl ester (L-NAME). Animals receiving L-NAME were given the drug ad libitum in their drinking water over a range of concentrations (10–250 mM), beginning 4 days prior to killing.

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Abbreviations: NOS, nitric oxide synthase; LPS, bacterial lipopolysaccharide; SIN-1, 3-morpholinosydnonimine *N*-ethylcarbamide; L-NAME, N^{ω}-nitro-L-arginine methyl ester; PB, phenobarbital; β-NF, β-naphthoflavone; 3-MC, 3-methylcholanthrene; AD, 4-androsten-3,17-dione; 7-PR, 7-pentoxyresorufin.

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¶Some of these results were presented at the British Pharmacological Society meeting in Dublin in July 1992 and published in abstract form (10).

NO-Generating System. Cultured aortic smooth-muscle cells were pretreated with LPS and rat interferon γ to induce nitric oxide synthase (NOS), and cytosol was prepared as described (12). NOS activity in the cytosol was 30–40 nmol/min per mg of protein. The influence of NO on microsomal P450 activity was assessed after incubation of rat liver microsomes (400 μg of protein) with NOS-containing cytosol (150–240 μg/ml of protein) and a mixture of NOS substrates and cofactors (12).

NOS Assay. NO production by induced smooth-muscle cell cytosol was assessed from the kinetics of oxidation of Fe^{2+}-myoglobin to metmyoglobin, as described (12). This method was also used for the assay of NO production by 3-morpholinosydnonimine *N*-ethylcarbamide (SIN-1).

Plasma Nitrate Assay. Plasma samples were diluted as necessary with 50 mM phosphate buffer (pH 7.5) to 120 μl, and protein was precipitated by adding 20 μl of 15% (wt/vol) trichloroacetic acid. After centrifugation at 10,000 × *g* for 10 min, samples were neutralized by adding 10 μl of 0.75 M NaOH and 20 μl of 50 mM phosphate buffer, pH 7.5. Nitrate was converted to nitrite and assayed by the method of Griess as modified by Schmidt *et al.* (13).

Cytochrome P450 Activity. 7-Ethoxycoumarin-*O*-deethylase activity was measured by the method of Greenlee and Poland (14), as modified by Rifkind *et al.* (15). CYP2B1/2 specific activity was assessed by 7-pentoxyresorufin (7-PR) O-dealkylation (16) and from 4-androsten-3,17-dione (AD) 16β-hydroxylation using corticosterone as an internal standard for recovery (17, 18).

Protein Assay. Protein concentration was measured by the dye-binding assay of Bradford (19) with bovine serum albumin as standard.

Chemicals. SIN-1 was from Cassella AG (Frankfurt, Germany), L-N^{ω}-methylarginine was synthesized as previously described (20). Rat recombinant interferon γ was from GIBCO. Tetrahydrobiopterin was from B. Schircks (Jona, Switzerland). 7α-, 16α-, and 6β-Hydroxyandrostenediones were from Steraloids (Croydon, U.K.). *Escherichia coli* LPS (serotype 0111:B4), L-NAME, and all other drugs and analytical reagents were from Sigma or BDH.

Statistical Analyses. Statistical analyses were done by using the nonpaired, one-tailed Student's *t* test and linear-regression analysis.

RESULTS

NO Inhibits Activity of Cytochrome P450 *in Vitro*. PB treatment of rats substantially induces the CYP2B1/2 in rat liver microsomes. These isoforms are important in metabolism of some drugs and environmental chemicals. A prototypical reaction catalyzed by CYP2B1/2 is the hydroxylation of AD at the 16β position (21). Fig. 1 shows that the rate of AD hydroxylation by hepatic microsomes from PB-treated rats is substantially inhibited by pretreatment with the NO-generating compound SIN-1. Inhibition by SIN-1 was both dose- and time-dependent, with 50% inhibition occurring after 5-min exposure to a flux of 100 pmol of NO per min. When SIN-1 spontaneously decomposes to produce NO, superoxide anion is also formed (22). Nevertheless, a specific role of NO in the inhibition by SIN-1 of AD hydroxylation by hepatic microsomes is indicated by our finding that the effect of SIN-1 was completely abolished by the NO scavenger Fe^{2+}-myoglobin. With 20 μM myoglobin, SIN-1 (2 mM) did not reduce AD metabolism significantly from control level (92 ± 5% of control, $n = 3$; $P = 0.2$), whereas without myoglobin significant suppression by SIN-1 was seen (70 ± 6%, $n = 3$; $P = 0.03$).

To examine the generality of NO as an inhibitor of cytochromes P450 *in vitro*, experiments were done with enzymatically derived NO. The cytosolic fraction of cytokine-activated rat aortic smooth-muscle cells (23) was used as a source of NOS, which could be activated by addition of substrates or terminated by the specific inhibitor N^{ω}-methyl-L-arginine. Brief preincubation of NOS with substrates and cofactors with cytochrome P450-enriched hepatic microsomes significantly inhibited (>60%) the cytochrome P450-dependent dealkylation of 7-ethoxycoumarin (Fig. 2). This finding was obtained with microsomes from each of two species, chicken and rat, and after chemical induction of cytochromes P450 by each of three agents, β-NF, 3-MC, and PB. NMA prevented NOS from inhibiting metabolism of 7-ethoxycoumarin in chicken liver and substantially diminished the inhibition in rat liver. Moreover, inhibition was not seen when L-arginine was excluded from the incubation mixture. Taken together, these findings imply that NO me-

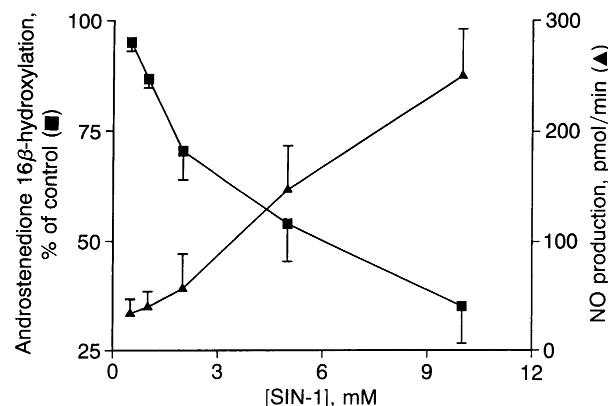

FIG. 1. NO production by SIN-1 and its inhibitory effect on cytochrome P450-dependent metabolism of AD. Microsomes from PB-treated rats were isolated and exposed to the indicated concentrations of SIN-1 for 10 min. AD was then added to a final concentration of 70 μM, and samples were incubated for another 5 min. Hydroxylated metabolites of AD were measured by HPLC, as described (18). The influence of SIN-1 on production of 16β-hydroxyandrostendione, a CYP2B1-mediated metabolism selectively induced by PB, is shown. Assay of NO synthesis from SIN-1 was measured by the myoglobin capture assay, as described in *Materials and Methods*. Values are means ± SEM ($n = 3$).

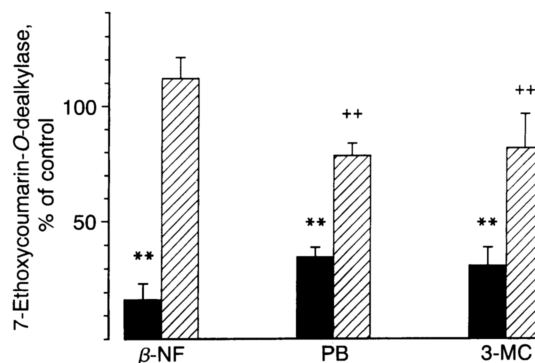

FIG. 2. Inhibitory effect of NO on cytochrome P450 activity in hepatic microsomes from chicken embryo and rat. Chicken eggs or rats were treated with the indicated cytochrome P450-inducing agents, PB, β-NF, or 3 MC. 7-Ethoxycoumarin-*O*-deethylase activity was measured during a 5-min interval with microsomes preincubated with NOS and cofactors alone (■) or with NOS, cofactors, and 10 mM N^{ω}-methyl-L-arginine (NMA) (▨) for 3 min (chicken) or 5 min (rat). During the preincubation period with the NO-generating system, NO production was 750–900 pmol/min. NMA inhibited this NO synthesis by >95%. Values are expressed as means ± SEM of the percentage activity seen without NOS pretreatment (control; n = 3–4). **, Significant inhibition of activity, relative to control (P < 0.01); ++, significant increase in activity, relative to treatment with NOS alone (P < 0.01).

diates the observed inhibition of cytochrome P450-dependent metabolism. The inhibition by NO of cytochrome P450 activity in livers of animals treated with PB and 3-MC, which induce different cytochrome P450 isoforms (4–6), suggests that NO inhibits the activity of cytochromes P450 derived from more than one species and molecular family.

Spectral Evidence for NO Binding to Cytochrome P450 Heme Iron *in Vitro*. To determine whether inhibition of cytochrome P450 by NO involves heme binding, spectral studies were done (Fig. 3). CO binding to dithionite-reduced microsomes produced a characteristic difference spectrum with an absorption maximum at 450 nm serving to define and quantitate cytochromes P450 (Fig. 3, curve 1). On the other hand, exposure of nonreduced microsomes to SIN-1 caused the immediate appearance of a peak at ≈436 nm, attributed to the Fe^{2+}-heme adduct of NO (curve 2). Subsequent reduction of the SIN-1-treated microsomes with dithionite caused an immediate shift in the 436-nm peak to 448–450 nm (attributed to the Fe^{2+}-heme adduct of cytochromes P450 with NO), as well as the appearance of a peak at 425 nm, representing Fe^{2+}-cytochrome b_5 (curve 3). The 448- to 450-nm peak increased in height, stabilizing after ≈5 min (curve 4). Similar spectral changes were observed with SIN-1, encased in a reservoir, prepared from gas-permeable silastic tubing, suggesting that the effects of SIN-1 are mediated by NO rather than either SIN-1 itself or nongaseous degradation products. Superoxide was not involved in these spectral changes, as appearance of the 448- to 450-nm peak was not altered by superoxide dismutase (200 units/ml, data not shown). Addition of CO to SIN-1-pretreated microsomes caused only a very small further increase in the 448–450 nm (curve 5), suggesting that NO effectively obstructs CO binding.

LPS-Mediated Overproduction of NO Inhibits Cytochromes P450 *in Vivo*. Treatment of rats with a 3-day course of PB caused an ≈30-fold elevation in the cytochrome CYP2B1/2 activity of hepatic microsomes, based on the ability of microsomes to dealkylate 7-PR (Fig. 4). In agreement with previous reports (9, 11, 24) that LPS attenuated PB-induced P450 activity, microsomal metabolism of 7-PR was diminished by 66 ± 11% in animals receiving a single dose (4 mg/kg i.p.) of LPS, 6 hr prior to killing. LPS treatment also elicited a large elevation of plasma nitrate. This LPS-induced nitrate elevation arises from NOS induction, as the elevation was suppressed by ≈90% in animals treated concurrently with L-NAME (>0.5 mmol of L-NAME consumed per rat during a 4-day period; see Fig. 4). Notably, L-NAME also significantly reduced the inhibition in CYP2B1/2 activity by LPS from 66 ± 11% to 28 ± 19% of control (P = 0.018). These findings implicate LPS-induced NO as a mediator of the decrease in cytochrome P450 activity. Additional support for this view comes from the strong correlation (r = 0.85) between plasma nitrate levels in all LPS-treated rats (with and without L-NAME) and the degree of inhibition of PB-induced hepatic cytochrome P450 activity (Fig. 5).

Fig. 6 shows that PB treatment significantly increased the hepatic microsomal content of cytochrome P450 and cytochrome b_5 (≈200% of control on a per mg of protein basis).

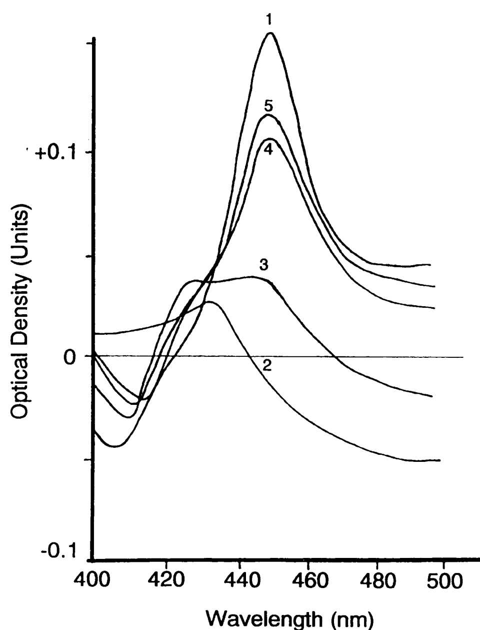

FIG. 3. Absorption spectra of microsomes from PB-treated rats after exposure to CO and NO. Curves: 1, a prototypic absorption spectrum of hepatic microsomes with a 450-nm peak after reduction by sodium dithionite and bubbling with CO; 2–5, sequential addition of 10 mM SIN-1 (5 min after addition, curve 2), sodium dithionite (1 min after addition, curve 3; 5 min after addition, curve 4), or CO (2 min after addition, curve 5).

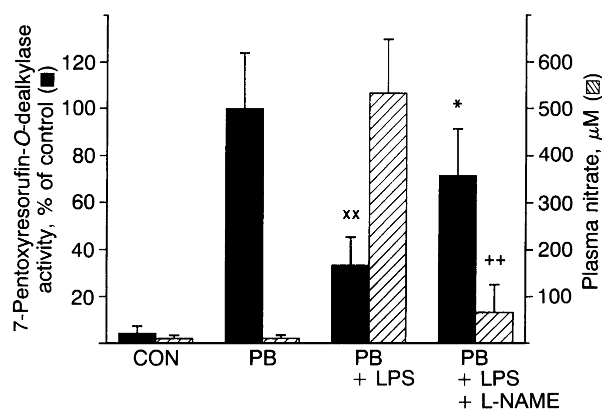

FIG. 4. Effect of LPS *in vivo* with and without the NOS inhibitor L-NAME, on PB-induced cytochrome P450-dependent metabolism and NO production. Groups of rats were treated with drugs, and hepatic microsomes were prepared as described. Microsomes were analyzed for their ability to catalyze dealkylation of 7-PR, and plasma was quantified for nitrate levels. Bars represent means ± SEMs (n = 5–17). xx, Significant decrease by LPS in 7-PR metabolism, relative to PB treatment alone ($P < 0.01$). *, Significant protection by L-NAME against the decrease in 7-PR metabolism from LPS ($P < 0.05$). ++, Significant protection by L-NAME against LPS-induced nitrate elevation ($P < 0.01$).

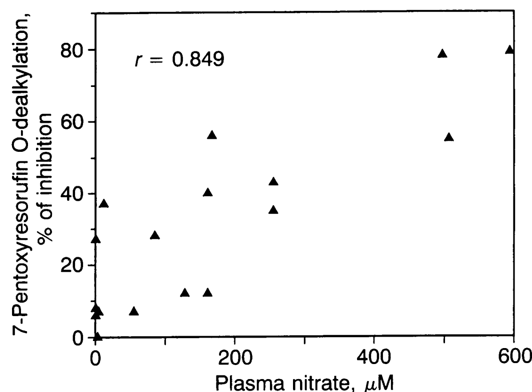

FIG. 5. Correlation between LPS-induced changes in plasma nitrate levels and inhibition of cytochrome P450-dependent metabolism. Rats were treated with LPS (2 mg/kg for 6 hr or 4 mg/kg for 6 or 15 hr) with or without L-NAME (n = 17). Microsomes were assayed for dealkylation of 7-PR, and plasma was assayed for nitrate levels. Each data point represents the mean of duplicate determinations on an individual rat. Linear-regression analysis indicates significant correlation between nitrate levels and 7-PR dealkylation (r = 0.845).

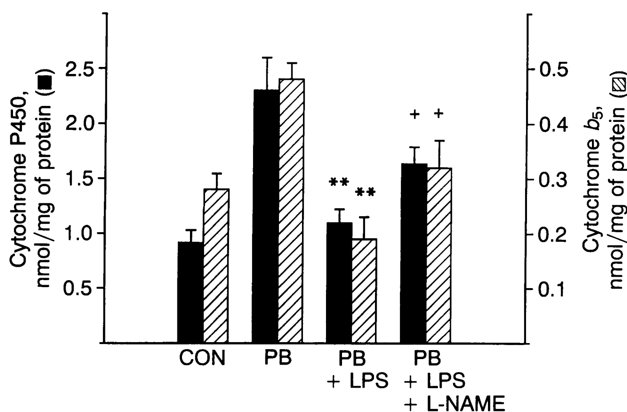

FIG. 6. Effect of treatment with LPS *in vivo*, with and without L-NAME, on induction of hepatic microsomal cytochromes P450 and b_5 elicited by PB. Groups of rats were treated with the indicated agents, as described, and liver microsomes were prepared. Bars depict means ± SEMs (n = 5–9). **, Significant reduction in PB-induced cytochrome content by LPS ($P < 0.01$); +, significant protection by L-NAME against the reduction in PB-induced cytochromes from LPS ($P < 0.05$). CON, control.

However, no increase was seen in microsomes of PB-treated rats exposed to LPS for only 6 hr before the end of a 3-day P450-induction period. Pretreatment of animals with L-NAME partially overcame the inhibitory effect of LPS on spectrally determined cytochrome content.

DISCUSSION

The liver has a greatly diminished capacity to metabolize endogenous substances and drugs after infection by viruses, bacteria, or fungi. A similar suppression of drug metabolism occurs after treatment of animals or hepatocytes in culture with immunoactivators, including cytokines and cytokine-releasing agents. The suppression of hepatic metabolism by immunoactivators arises from a reduced activity of the cytochrome P450 mixed-function oxidase system (8, 9). The phenomenon is important in that immunostimulant-induced suppression of metabolism by cytochromes P450 prolongs the duration and intensity of action of drugs and endogenously produced substances *in vivo* (8, 9). Here, we show that overproduction of NO is involved in the suppression of cytochrome P450 activity by LPS.

Arginine-derived NO is a secretory product of mammalian cells, where it serves diverse functions in intercellular communication (1–3). For cell-signaling, NO is produced by a low-output enzyme in neurons and endothelial cells; this enzyme is activated by transient elevations in intracellular Ca^{2+}. Immunostimulants induce the expression of a high-output, Ca^{2+}-independent isoform of NOS in many cell types, including hepatocytes and Kupffer cells (25–27). Before the discovery of NO as a biosignaling molecule, NO was known to bind with great avidity to the heme iron of hemoproteins (2, 3), including cytochromes P450 (28). We and Wink *et al.* (29) have shown that NO, in supraphysiological concentrations, will impair cytochrome P450-mediated metabolism by isolated hepatic microsomes. The unusual and most important aspect of our present study is the discovery that NO can be produced *in vivo* in sufficient quantities to inhibit P450 function. Indeed, we found that the NOS inhibitor L-NAME significantly prevents the inhibition of cytochrome P450 function by LPS. The degree of P450 inhibition by LPS directly correlated with plasma nitrate levels. Moreover, the ability of L-NAME to prevent P450 inhibition correlated with its ability to prevent the LPS-induced elevation in plasma nitrate. These findings strongly support the concept that cytochromes P450 are inactivated by NO levels produced *in vivo*, after NOS induction by LPS. Since the first report in 1953 showing that drug-induced stimulation of host defense down-regulates drug metabolism (24) (a finding that predated the discovery of cytochromes P450 by a decade), investigators have sought the mechanism of this effect. Although many explanations have been postulated, the mechanism has remained obscure. On the basis of our results and findings that immunostimulatory agents that attenuate drug metabolism (9, 10) also induce NOS (2, 3), we propose that NO is the principal effector of the generalized suppression of hepatic drug metabolism associated with immunoactivation.

Inactivation of cytochromes P450 by NO was observed *in vitro* with microsomes from both chicken and rat and occurred with the several substrates and inducing agents tested. Although these results suggest that NO may be a nonspecific inhibitor of all cytochromes P450, NO could affect cytochrome P450 isoforms differently, based on differences in accessibility of heme to NO. Although the work presented here has focused on inducible P450 isoforms, our preliminary results also indicate that constitutively expressed cytochromes P450 are susceptible to inactivation by NO. The ability of L-NAME substantially to attenuate both LPS-induced NO production and the decrease in cytochrome P450 activity implies that LPS-induced NO overproduction is a necessary event for the ensuing loss of cytochrome P450 activity and content. Although L-NAME did not fully prevent the LPS-induced decrease in cytochrome P450 activity in rat liver microsomes, we found that L-NAME itself can inhibit CYP2B1/2 activity *in vitro* (IC_{50} for 7-PR-*O*-dealkylase = 0.7 mM; unpublished data). Moreover, high concentrations of L-NAME bind ferrous iron and interfere with cytochrome *c* reduction (30). Thus, direct inhibition of cytochrome P450 activity by L-NAME may explain the incomplete protection against the P450 suppressive effect of LPS in rat liver.

Recent findings by Wink *et al.* (29) that *in vitro* exposure of rat hepatic microsomes to NO inhibits 7-ethoxyresorufin *O*-deethylation and benzyloxyresorufin *O*-dealkylation are complementary to our findings. We also found inhibition of 7-ethoxyresorufin as well as 7-PR-*O*-dealkylase with NO but, in addition, found that NO interacts directly with 7-ethoxyresorufin and 7-PR, causing substrate loss and complicating interpretation of findings with these substrates (unpublished data).

Our spectrophotometric studies and those of others (28) clearly show that NO binds to both ferric and ferrous hemoproteins. The finding of an NO difference spectrum with a 450-nm peak shows that NO interacts with the intact cytochrome P450 hemoprotein; this is consistent with the finding of Wink *et al.* (29) that part of the inhibition of mixed-function oxidase activity by NO *in vitro* was reversible, as such reversibility would also require intact P450. The capacity of NO to bind to P450 indicates that our observed decrease in total P450 could reflect masking of CO binding by NO (P450 inactivation) rather than true decrease in P450 content.

In addition to interacting with intact P450, NO could impair P450 function by enhancing degradation of the heme or apoprotein moieties of P450. With respect to the first possibility, dissociation of the heme from holocytochrome P450 has been postulated as a mechanism for LPS suppression of P450 function (31). It is noteworthy that LPS treatment causes nitrosylation of hemoglobin (32, 33), making it likely that the hepatic cytochromes that are also hemoproteins would be similarly modified. Alkylation or oxidative damage can destroy heme and impair P450 function. It can also lead to selective degradation of apoprotein portions of different P450 isoforms (34). Thus, nitrosylation could enhance the degradation of either the heme or apoprotein moieties of

P450. Our findings also reveal that another major microsomal hemoprotein, cytochrome b_5, is depressed by NO. The same mechanisms postulated to explain suppression of P450 could apply to cytochrome b_5. Electron paramagnetic resonance studies would be useful to determine the extent to which cytochromes P450 and cytochrome b_5 are nitrosylated in animals treated with LPS.

Diminished transcription of cytochrome P450 genes by immunostimulants and cytokines has also been reported (35). Nitrosylation of transcriptional regulatory factors by NO could, for example, down-regulate transcription of cytochrome P450 mRNA. In this regard, it is noteworthy that the iron-response element-binding protein contains an iron–sulfur complex homologous to the one present in aconitase (36), a known target of NO (37). Similarly, thiol groups of cysteine are susceptible to nitrosylation by NO (38) and are necessary for transcriptional activation by factors requiring metal chelation—e.g., zinc-finger proteins. Thus, NO could inhibit cytochrome P450 by disrupting its transcriptional activation. Under our experimental conditions, however, it is more likely that the LPS effect is mediated by inactivation of preexisting cytochromes than by inhibiting new cytochrome synthesis because LPS was administered only during the last hours of a 72-hr induction period, when P450 content would already be approaching maximum (17).

We propose the following events to explain our findings and accommodate prior knowledge of how LPS inhibits hepatic cytochrome P450-dependent metabolism: (*i*) LPS induces the release of intermediary cytokines, which, in turn, induce NOS activity in Kupffer cells and hepatocytes; (*ii*) NO binds to heme iron in cytochromes P450 and prevents oxygen binding, thereby blocking enzyme activity; (*iii*) NO may also enhance degradation of cytochromes P450 by nitrosylation of heme or thiols in P450 apoprotein or impair transcriptional activation of P450. This model is consistent with evidence that LPS-induced suppression of hepatic metabolism *in vivo* is prevented by dexamethasone (39), protein-synthesis inhibitors (9), and antiinterleukin 1 antiserum (39), each of which can prevent NOS induction in cells (2, 40). This model is also consistent with evidence that agents that stimulate reticuloendothelial cell function stimulate production of a low-molecular-weight and diffusible factor (characteristics of NO) that inhibits P450 activity (41). Whereas LPS directly induces NOS in Kupffer cells, products secondarily released from Kupffer cells can induce NOS in hepatocytes (27). Thus, hepatocytes can become both the source and the target of the NO that attenuates cytochrome P450-dependent metabolism. Generation of NO by hepatocytes is not merely a cell culture artifact because LPS treatment *in vivo* activates NO synthesis in subsequently isolated rat hepatocytes (27).

In summary, cytokine-induced overproduction of NO could explain attenuation of activity, content, and transcription of cytochromes P450 by a diverse array of immunostimulants. NO-mediated suppression of cytochromes P450 could have important clinical bearing on the pharmacotherapy of patients who have infections or are undergoing cancer chemotherapy with cytokines.

We thank Ivana Vojnović and Charis Lee for expert technical assistance. This work was supported by Grants HL46403 (S.S.G.) and ES03606 (A.B.R.) from the National Institutes of Health and a grant from Glaxo Group Research Ltd.

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