Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 3559–3563, April 1994
Medical Sciences

Inhibition of cytochromes P4501A by nitric oxide

(V79 fibroblasts/hepatocytes/cytokines/inflammation)

JOSEF STADLER*†, JÖRG TROCKFELD*, WOLFGANG A. SCHMALIX‡, THOMAS BRILL§, J. RÜDIGER SIEWERT*, HELMUT GREIM‡, AND JOHANNES DOEHMER‡

*Chirurgische Klinik und Poliklinik, ‡Institut für Toxikologie und Umwelthygiene, and §Institut für Experimentelle Chirurgie, Technische Universität München, Ismaninger Strasse 22, 81675 Munich, Germany

Communicated by Ronald W. Estabrook, January 3, 1994 (received for review October 18, 1993)

ABSTRACT **Inflammatory stimulation of the liver leads to the induction of nitric oxide (NO) biosynthesis. Because NO binds to the catalytic heme moiety of cytochromes P450 (CYPs), we investigated whether NO interferes with specific CYP-dependent metabolic pathways. In a first experimental approach V79 Chinese hamster cells genetically engineered for stable expression of rat and human CYP1A1 and -1A2 were used. Incubation with the NO donors sodium nitroprusside and *S*-nitrosylacetylpenicillamine led to a concentration-dependent inhibition of all four CYP enzymes. CYP1A1 was more sensitive to the inhibitory effect of NO than CYP1A2. In the second part of the study, endogenous NO synthesis was induced in rat hepatocytes by incubation with a mixture of cytokines and endotoxin. Concurrently, as NO production in hepatocytes increased within 24 hr, a decrease in CYP1A1-dependent benzo[*a*]pyrene turnover was observed to almost undetectable levels. The competitive inhibitor of NO synthesis, N^G-monomethyl-L-arginine, was able to significantly restore CYP1A1 activity in the presence of cytokines and endotoxin. Inhibition of hepatocellular CYP activity by NO was predominantly due to a direct effect on the enzymes. However, NO-dependent inhibition of CYP expression at a transcriptional level was also demonstrated. Our results indicate that inhibition of NO biosynthesis in patients suffering from systemic inflammatory response syndromes may help to restore biotransformation capacity of the liver.**

Hepatocellular dysfunction is a detrimental consequence of the metabolic response of the liver to prolonged inflammatory stimulation. Characteristic features are suppression of synthetic performance and a profound inhibition of xenobiotic biotransformation (1, 2). Therapy is not available because the molecular basis of these phenomena remains to be elucidated. However, a better understanding of the pathophysiology of the inflamed liver may result from the discovery of L-arginine-dependent nitric oxide (NO) production in hepatocytes and nonparenchymal liver cells (3, 4). As in many other cell types, an inducible NO synthase (iNOS) was identified in hepatocytes upon stimulation with cytokines and endotoxin (5, 6). Induction of iNOS was also observed in the course of parasitic infections, such as malaria (7). Recently, the gene encoding iNOS has been cloned from human hepatocytes in full length for heterologous expression (8).

NO exerts a plethora of biologic functions (9). Many of these effects are based on modulation of enzyme activity through binding of NO to prosthetic iron complexes. In this context it is interesting that NO was used for years as a spin-label probe to investigate the role of heme groups in the catalytic centers of cytochrome P450 (CYP) enzymes (10). Consequently, it was demonstrated that NO inhibits CYP-dependent reactions when microsomal preparations were exposed to NO (11). CYP enzymes, also referred to as microsomal monooxygenases, catalyze oxidative key reactions in the biotransformation of xenobiotics (12). Therefore, inhibition of these enzymes would severely affect hepatocellular detoxication. However, suppression of CYP-dependent metabolism has not yet been demonstrated for endogenously produced NO. Investigations of other effects of NO, particularly the inhibition of mitochondrial respiration, revealed that major differences between exogenously applied and endogenously produced NO can be observed (13). In light of these observations hepatocytes seem to have the capacity to mostly resist metabolic inhibition by endogenous NO. In addition, the effects of NO on biotransformation are clouded by the fact that transcriptional suppression of CYP expression was described after inflammatory stimulation (14, 15). The responsible mediators were found to be the same that induce hepatocellular iNOS.

In an attempt to clarify this complex situation, a 2-fold experimental approach was designed. In the first set of experiments, V79 Chinese hamster cells genetically engineered for stable expression of CYPs were exposed to exogenous NO to study the immediate effects of NO on specific CYP activities. The second approach made use of freshly prepared rat hepatocytes, in which endogenous NO synthesis was induced by incubation with cytokines and endotoxin. In these hepatocytes CYP-dependent metabolism was tested, and specific CYP expression as well as relative concentrations were determined by RNA and immunoblot analyses.

MATERIALS AND METHODS

Recombinant rat interferon γ (IFN-γ) was from GIBCO/Life Technologies, recombinant murine tumor necrosis factor α (TNF-α) and recombinant human interleukin 1 (IL-1) were from Genzyme. Polyclonal rabbit anti-CYP 1A1 antiserum was from Oxygene (Dallas), and other reagents were obtained from Sigma or other commercial sources as indicated. Monoclonal anti-CYP1A2 antibodies (D15) were provided by F. Wächter (Sandoz Pharmaceutical, Basel) and polyclonal rat anti-CYP2E1 antiserum was from M. Ingelman-Sundberg (Karolinska Institute, Stockholm).

Cell Cultures. V79 Chinese hamster cells were genetically engineered for stable expression of CYP enzymes (16). For the reported experiments recombinant V79 cell lines expressing rat and human CYP1A1 and -1A2 were used (17–20). Cells were maintained in Dulbecco-Vogt's modified Eagle's me-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NO, nitric oxide; iNOS, inducible NO synthase; NMA, N^G-monomethyl-L-arginine; IFN-γ, interferon γ; TNF-α, tumor necrosis factor α; IL-1, interleukin 1; LPS, lipopolysaccharide; CM, cytokine mixture with LPS; SNP, sodium nitroprusside; SNAP, *S*-nitrosoacetylpenicillamine; CYP, cytochrome P450; AHH, aryl hydrocarbon hydroxylase; EROD, 7-ethoxyresorufin-*O*-dealkylase.

†To whom reprint requests should be addressed.

dium (DMEM; GIBCO) supplemented with 10% fetal calf serum, penicillin (100 units/ml) and streptomycin (100 μg/ml).

Hepatocytes were isolated from male Sprague–Dawley rats weighing 200–300 g (Charles River WIGA, Kisslegg, F.R.G.). The liver cells were harvested with an *in situ* collagenase (type IV; Sigma) perfusion technique, and the hepatocytes were purified by repeated differential sedimentation at 50 × *g* for 2 min (21). The cell cultures were maintained in William's medium E (GIBCO)/10% fetal calf serum/penicillin (100 units/ml)/streptomycin (100 μg/ml)/L-glutamine (2 mmol/liter)/insulin (1 μmol/liter). Cell densities were adjusted to 4×10^5/ml, and the incubations were continued for 24 hr.

Application of Exogenous NO and Induction of Endogenous NO Synthesis. Exogenous NO was applied to V79-derived cell lines by two different substances, which spontaneously release NO. The first NO donor was sodium nitroprusside (SNP; Sigma). The second NO donor, *S*-nitrosoacetylpenicillamine (SNAP), was synthesized according to Field *et al.* (22). SNP readily dissolves in water, but SNAP was dissolved in dimethyl sulfoxide before further dilution in reaction buffers. Because dimethyl sulfoxide suppressed both rat and human CYP1A2 activity by itself, methanol was used as a solvent of SNAP for the CYP1A2 reactions. The release of NO, as measured by accumulation of nitrite, was identical in both solvents.

Endogenous NO synthesis was induced in rat hepatocytes by incubation with a combination of recombinant murine TNF-α (500 units/ml), recombinant human IL-1 (5 units/ml), recombinant rat IFN-γ (100 units/ml), and lipopolysaccharide (LPS; 10 μg/ml from *Escherichia coli* 111:B_4; Sigma). This cytokine mixture in combination with LPS will be referred to hereafter as CM. N^G-monomethyl-L-arginine (NMA; Sigma) was used as a competitive inhibitor of L-arginine-dependent NO biosynthesis.

Determination of Nitrite Concentrations. As an oxidation product of NO biosynthesis, nitrite was determined spectrophotometrically at 540 nm, based on a diazotization assay, generally referred to as a Griess reaction (23). In this reaction the adduct of nitrite and sulfanilic acid interacts with *N*-(1-naphthyl)ethylenediamine to form a purple azo dye, which is finally measured.

Enzyme Assays. Aryl hydrocarbon hydroxylase (AHH) activity of rat and human CYP1A1 enzymes was determined by measuring the fluorescence of the hydroxylated metabolites of benzo[*a*]pyrene with excitation at 396 nm and emission at 522 nm (24); 3-OH-benzo[*a*]pyrene was used as a reference. For the enzyme assay 0.4 mg of V79 cell protein was added to 1 ml of reaction buffer containing 50 μmol Tris·HCl (pH 7.6), 3 μmol of $MgCl_2$, 0.6 μmol of NADPH, and SNP or SNAP in concentrations ranging from 0.01 to 10 mM. After 30 min of exposure to the NO donors on ice the reaction was started by the addition of 100 nmol of benzo[*a*]pyrene and maintained for 30 min at 37°C. The reaction was stopped by addition of propanol/hexane, 1:3 (vol/vol) (4 ml).

CYP1A2 activity was determined by the 7-ethoxyresorufin-*O*-dealkylase (EROD) dealkylation to resorufin, which was measured spectrofluorometrically at an excitation of 550 nm and an emission of 585 nm. V79 cell protein (0.4 mg) was added to 1.25 ml of reaction mixture containing Tris·HCl (pH 7.6) at 50 μmol/ml, $MgSO_4$ at 5 μmol/ml, NADPH at 0.4 μmol/ml, Dicumarol at 10 nmol/ml, and SNP or SNAP at concentrations ranging from 0.01 to 10 mM. After 30 min of exposure to NO on ice the reaction was started with 2 nmol of ethoxyresorufin and stopped after 30 min of further incubation at 37°C by the addition of 2.5 ml of methanol. The total time of exposure to the NO donors was 60 min for both AHH and EROD reactions. During this time NO release was linear. All reagents for the AHH and the EROD assays were from Sigma.

AHH activity was also assayed in rat hepatocyte cultures (25). For induction of CYPs of the 1A subfamily one-half of the cultures was incubated with β-naphthoflavone at 50 nmol/ml. After an incubation period of 24 hr the medium was changed to fresh medium containing benzo[*a*]pyrene at 20 nmol/ml as a substrate for the AHH reaction. The conversion to 3-OH-benzo[*a*]pyrene was determined 3 hr later spectrofluorometrically, as described above.

Immunoblot Analysis. Relative concentrations of CYP1A1, -1A2, and -2E1 from rat hepatocytes were determined after electrophoretic separation of cellular homogenates (SDS/PAGE) according to Laemmli (26), transfer onto Immobilon protein-binding membrane (Millipore), and immunological detection of proteins by using antisera directed against the enzyme isoforms of interest (27). Specifically bound antibodies were detected with appropriate horseradish peroxidase-conjugated secondary antibodies and the enhanced chemoluminescence (ECL) method using an ECL-kit (Amersham). Relative intensities of bands were quantitated by laser densitometry with the UltroScan apparatus (Pharmacia LKB).

RNA Blot Analysis. To determine relative steady-state concentrations of CYP1A1 mRNAs in rat hepatocytes, total RNA was prepared by lysis of cells in a buffer containing 5 M guanidium isothiocyanate, 500 mM 2-mercaptoethanol, and 50 mM sodium acetate (pH 5.5). The lysate was layered on a 2.5-ml cushion of CsCl solution in 10 mM Tris·HCl (pH 7.0) and 1 mM EDTA with a density of 1.8 g/cm^3 and centrifuged at 110,000 × *g* for 16 hr (28). Pelleted RNA was dissolved in H_2O, extracted twice by phenol, ethanol precipitated, and resuspended in H_2O at a concentration of 1 μg/μl. Twenty micrograms of total RNA was glyoxylated and electrophoresed on a 1.5% agarose gel and blot-transferred onto GeneScreenPlus nucleic acid-binding membranes (Schleicher & Schüll). Hybridizations were done in 1 M NaCl/Denhardt's solution/salmon sperm DNA at 250 μg/ml/50 mM Tris, pH 7.0/50% (vol/vol) deionized formamide at 42°C for 18 hr. DNA probes were labeled with [α-^{32}P]dCTP (specific activity 3000 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) by random priming. Hybridized membranes were washed twice at room temperature in 2× standard saline citrate (SSC), twice for 30 min at 65°C in 2× SSC/1% SDS, and twice for 15 min at room temperature in 0.1× SSC. Autoradiography was done by exposure to Kodak X-Omat film at −70°C in the presence of intensifying screens.

Protein Determination. Protein concentrations were determined according to Bradford (29) using the Bio-Rad assay solution and bovine serum albumin as a standard.

Statistical Analysis. Values are given as means ± SDs representing at least three experiments. The significance of differences was determined with a two-tailed Student's *t* test.

RESULTS

The effect of the NO donor SNP on specific CYP450 enzyme activity of members of the 1A subfamily is shown in Fig. 1. For these experiments genetically engineered V79-derived cell lines, which functionally express CYP1A1 and CYP1A2 of both rat (Fig. 1*A*) and human (Fig. 1*B*) origin, were used. Incubation with SNP for 1 hr led to a concentration-dependent inhibition of all four tested enzymes. Release of NO from SNP, as well as from SNAP, was linear over the whole period (data not shown). The CYP1A1 was more susceptible to the inhibitory effect of SNP than CYP1A2 and showed an almost linear response to logarithmically increasing SNP concentrations. This difference was more pronounced with rat than with human enzymes. Exposure to SNAP also caused a concentration-dependent decrease in the activity of all four enzymes (Fig. 2). Again, the CYP1A1

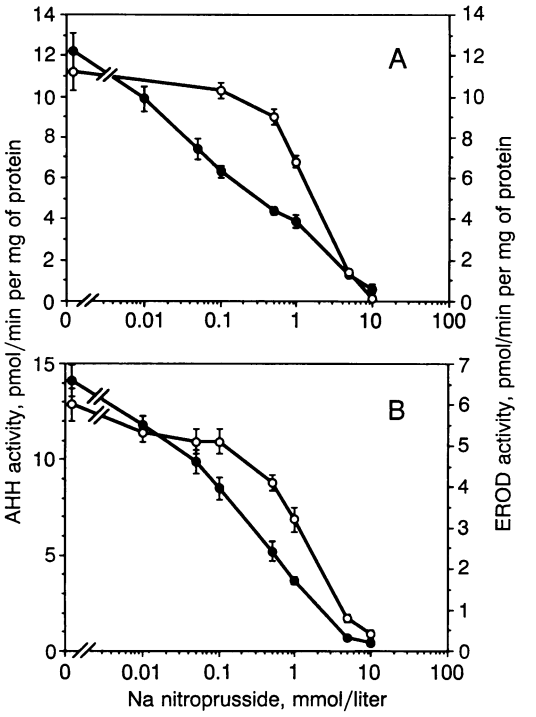

FIG. 1. Concentration-dependent inhibition of CYP1A1 (●) and CYP1A2 (○) by the NO donor SNP. Cellular homogenates of V79-derived cell lines, which constitutively express rat (*A*) or human (*B*) CYP enzymes, were incubated for 60 min with SNP in logarithmically increasing concentrations. For the last 30 min benzo[*a*]pyrene or ethoxyresorufin were added to test for AHH activity of CYP1A1 or for EROD activity of CYP1A2. Concentrations of the hydroxylated metabolites of benzo[*a*]pyrene and of resorufin, as products of the AHH and EROD reactions were determined spectrofluorometrically.

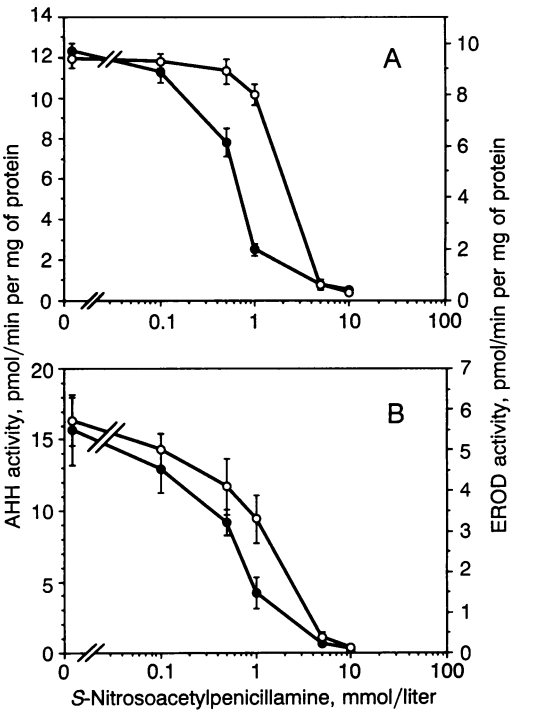

FIG. 2. Concentration-dependent inhibition of CYP1A1 (●) and CYP1A2 (○) enzymes of rat (*A*) and human (*B*) origin by the NO donor *S*-nitrosoacetylpenicillamine. For details of the experiments, see the legend to Fig. 1 and the text.

activity was more suppressed than that of CYP1A2. As described for SNP, this difference became more obvious in rat enzymes. However, the qualitative response of CYP1A1 and -1A2 to SNAP was almost identical, leading to similar courses of the inhibitory profiles.

To study the effects of endogenously produced NO, rat hepatocytes were used for further experiments. Incubation of the hepatocytes with CM led to the induction of NO biosynthesis. After 24 hr extensive NO production was seen, which was measured as the accumulative concentration of the oxidation product nitrite in the culture supernatants (Table 1). Under treatment with β-naphthoflavone CM-induced NO biosynthesis was further elevated, reaching nitrite levels >20 times higher than that of untreated control cells. NO production was almost completely suppressed by the addition of NMA to the incubation medium. In the 3-hr reaction phase, in which the metabolism of benzo[*a*]pyrene was tested, NO production was maintained at a high level, although CM was not present.

Because AHH activity of CYP1A1 was more susceptible to inhibition by exogenous NO, the effect of endogenous NO on AHH-dependent benzo[*a*]pyrene metabolism was determined. Under treatment with CM benzo[*a*]pyrene, turnover was reduced to 13% of the turnover by cells incubated in control medium (Table 2). Incubation with β-naphthoflavone led to a 4-fold increase of AHH activity in unstimulated control cells. However, in CM-treated cells this difference was totally blunted, and the activity ran up to only 4% of β-naphthoflavone-induced controls. The influence of NO production was demonstrated by the fact that inhibition of NO production by NMA significantly reversed the inhibition of benzo[*a*]pyrene turnover in CM-treated cells. However, NMA was not able to fully restore AHH activity, especially in β-naphthoflavone-treated cells in which only 34% of benzo[*a*]pyrene turnover of controls was reached.

Suppression of enzyme activity is principally due to either functional inhibition or to a decrease in enzyme content. To determine the relative amounts of specific CYP enzymes immunoblot analysis was performed by using β-naphthoflavone-treated hepatocytes. These experiments demonstrated slightly, but significantly, weaker bands of CYP1A1 and CYP1A2 under the influence of CM (Fig. 3). Quantification by laser densitometry showed a decrease to 61% of controls in the case of CYP1A1 and to 71% of controls looking at CYP1A2. CM-mediated down-regulation of protein concentration was inhibited by NMA only in the case of CYP1A1. Interestingly, no detectable differences were found for CYP2E1 throughout all incubations. According to these findings the inhibition of benzo[*a*]pyrene turnover by endog-

Table 1. NO biosynthesis by rat hepatocytes measured as accumulated nitrite concentrations in culture supernatants

Culture conditions		Nitrite production, nmol per 10^6 cells	
CM	NMA	Without induction	β-Naphthoflavone
		24-Hr induction period	
−	−	17.9 ± 13.7	16.3 ± 11.8
−	+	3.7 ± 2.1	4.3 ± 1.8
+	−	288.1 ± 53.1	348.3 ± 23.1*
+	+	12.4 ± 2.2	10.8 ± 3.1
		3-Hr benzo[*a*]pyrene metabolism period	
−	−	7.7 ± 2.9	10.7 ± 7.0
−	+	4.9 ± 1.5	5.6 ± 2.1
+	−	56.9 ± 7.7	70.9 ± 8.1*
+	+	7.0 ± 2.1	7.9 ± 3.0

CM was IFN-γ at 100 units/ml/TNF-α at 500 units/ml/IL-1 at 5 units/ml/LPS at 10 μg/ml. NMA and β-naphthoflavone were added at concentrations of 0.5 μmol/ml and 50 nmol/ml, respectively.
*$P \leq 0.05$ vs. CM without β-naphthoflavone.

Table 2. Endogenously produced NO inhibits hepatocellular metabolism of benzo[*a*]pyrene, a parameter of AHH activity

Culture conditions		Benzo[*a*]pyrene turnover, pmol/min per 10^6 cells	
CM	NMA	Without induction	β-Naphthoflavone
−	−	4.5 ± 1.4	18.6 ± 0.9
−	+	4.9 ± 0.8	19.0 ± 2.3
+	−	0.6 ± 0.2*	0.7 ± 0.2*
+	+	3.1 ± 0.6**	6.3 ± 1.2**

After a preincubation period of 24 hr for the induction of maximal NO biosynthesis the incubation medium was changed for fresh medium containing benzo[*a*]pyrene at 20 nmol/ml. Three hours later the concentration of hydroxylation products was determined spectrofluorometrically. CM was IFN-γ at 100 units/ml/TNF-α at 500 units/ml/IL-1 at 5 units/ml/LPS at 10 μg/ml. NMA and β-naphthoflavone were added at concentrations of 0.5 μmol/ml and 50 nmol/ml, respectively. *, $P \leq 0.01$ vs. untreated cells; **, $P \leq 0.01$ vs. CM-treated cells.

enous NO must have been predominantly due to functional inhibition of CYP activity, such as demonstrated in the experiments with exogenous NO.

NO biosynthesis was shown to suppress hepatocellular protein synthesis at a posttranscriptional level (30). For this reason it was interesting to correlate CYP1A1 protein and mRNA levels as detected by immuno and RNA blotting. Again, β-naphthoflavone-treated hepatocytes were used. Similar to the findings in the immunoblots, a decrease of CYP1A1 mRNA was detected on RNA blots under the influence of CM (Fig. 4). However, the degree of reduction of mRNA was strikingly more pronounced than the reduction in protein levels. Reduction of CYP1A1 mRNA was >80% (Fig. 4, lane CM), whereby the reduction of CYP1A1 protein was only 39% (Fig. 3, lane CM). Suppression of CYP1A1 mRNA was almost fully prevented in the presence of NMA (Fig. 4, lane CM/NMA).

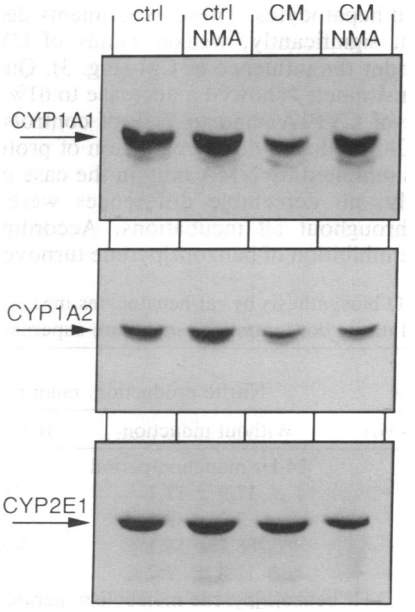

FIG. 3. Immunoblot analysis of the relative content of CYP1A1, CYP1A2, and CYP2E1 enzymes of rat hepatocytes after 24 hr of specific *in vitro* treatment. For maximal induction of CYP enzymes of the 1A subfamily β-naphthoflavone at 50 nmol/ml was added to all incubations. The analysis was done in three parallel sets with 50 μg of hepatocyte protein per lane. ctrl, Untreated cells; ctrl/NMA, 0.5 mM NMA was added; CM, IFN-γ/TNF-α/IL-1/LPS; CM/NMA, CM plus 0.5 mM NMA.

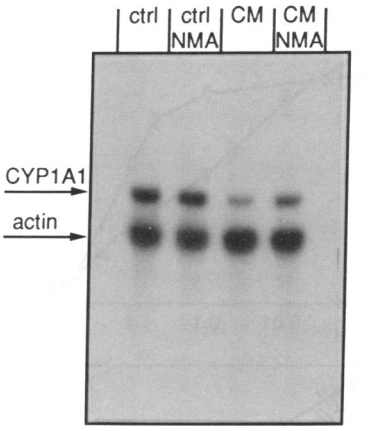

FIG. 4. RNA blot analysis of the relative content of mRNA of CYP1A1 of rat hepatocytes after 24 hr of specific *in vitro* treatment. For maximal induction of mRNA of CYP enzymes of the 1A subfamily β-naphthoflavone at 50 nmol/ml was added to all incubations. The analysis was done in three sets with 20 μg of total RNA per lane. ctrl, Untreated cells; ctrl/NMA, 0.5 mM NMA added; CM/NMA, CM plus 0.5 mM NMA.

DISCUSSION

NO can regulate the activity of important enzymes, such as the soluble guanylate cyclase or the enzyme complexes of the mitochondrial electron transport chain (31, 32). In this study it was demonstrated that NO affects hepatocellular biotransformation by inhibiting CYP enzyme activity.

Due to the complex regulation of CYP metabolism it is difficult to investigate effects on specific CYP enzymes using hepatocytes or other cells from natural sources. For such purposes genetically engineered V79-derived cell lines are ideal tools (33). With V79-derived cell lines constitutively expressing rat and human CYP1A1 and CYP1A2, it was demonstrated here that exogenously applied NO inhibits the activity of all four enzymes in a concentration-dependent manner. It was also shown that the susceptibility of CYP1A1 to the inhibitory effects of the NO donors is higher than that of CYP1A2. This finding confirms experiments using microsomal preparations, which suggested differences in the extent of NO-dependent inhibition of distinct CYP activities (11). There was also a qualitative difference of the inhibitory profiles after SNP or SNAP treatment. This problem may be attributable to the fact that SNP releases other reactive molecules such as cyanide in addition to NO (34).

In former experiments we had seen considerable differences between the effects of exogenously applied NO and endogenously produced NO on hepatocellular metabolism (13). The induction of iNOS by CM seemed to initiate unknown mechanisms that protected hepatocytes from metabolic inhibition by NO. Therefore, it was crucial to confirm the results obtained by application of exogenous NO to V79 cells for endogenously produced NO in hepatocytes. After induction of NO biosynthesis with CM a dramatic decrease of hepatocellular CYP-dependent benzo[*a*]pyrene turnover was seen, which was significantly reversed by the NO synthesis inhibitor NMA. By immunoblot analysis it was demonstrated that the 95% suppression of CYP metabolism was predominantly due to a direct inhibitory effect of NO on the enzyme activity rather than a decrease of enzyme concentrations, which reached maximally 40%. This set of experiments showed that endogenous NO has the same direct inhibitory effect on CYP activity as exogenously applied NO.

At this point it is not absolutely clear what the molecular basis of the inhibitory effect of NO on CYP enzymes really is. It is most likely that inhibition of enzyme activity is from binding of NO to the heme group in the catalytic center. In

this context it was shown that NO binds to CYP heme groups with high affinity (10) and that other hemoproteins, such as the cyclooxygenase or the lipoxygenase, are also inhibited by NO (35, 36). However, in a former study we have demonstrated by electron paramagnetic resonance (EPR) that predominantly nonheme iron–nitrosyl complexes are formed by NO in hepatocytes (37). EPR spectra of biological materials are composed of many different individual signals. Therefore, it is conceivable that the CYP heme signal only plays a minor role and does not contribute to the overall EPR signal to a detectable degree. On the other hand, it still may be speculated that functional inhibition of CYPs by NO is not attributable to heme binding but is attributable to other effects on the enzymes, such as oxidation of critical amino acids within the molecules (11). It is also interesting to note that NMA did not fully prevent inhibition of hepatocellular CYP activity by CM-induced NO release. The nature of this NO-independent effect remains to be uncovered.

In accordance with previous reports (15, 38) a decrease of CYP1A1 mRNA expression after CM treatment was observed. However, in the present study this was shown to be also NO dependent because NMA could significantly upregulate CYP1A1 mRNA. There was no suppression of actin mRNA, showing that this finding is not based on unspecific effects of NO like a decrease of cell viability. The difference between the strong effect of NO on CYP1A1 mRNA and the weak suppression of CYP1A1 concentration indicates that the CYP1A1 enzyme is more stable than the corresponding mRNA. Therefore, chronic NO exposure would probably lead to a more pronounced decrease of enzyme concentrations. In another study hepatocyte albumin and fibrinogen synthesis was found to be inhibited by NO at a posttranscriptional level (30). In summary, these different reports suggest that NO influences protein synthesis at various levels. Furthermore, the inhibitory effect on protein synthesis seems to include only certain proteins. As presented here CYP2E1 concentrations were not affected by the CM at all.

In conclusion, it was shown that inhibition of CYP1A1 activity of rat hepatocytes by incubation with a combination of proinflammatory cytokines and LPS is primarily due to functional inhibition by NO and, to a lesser degree, to suppression of enzyme expression at a transcriptional level. These findings may help to understand the problem of impaired detoxification by the inflamed liver and could potentially lead to therapeutic interventions because human hepatocytes have been shown to produce large amounts of NO under inflammatory stimulation (8, 39).

This work was supported by the Deutsche Forschungsgemeinschaft Bonn (Grant STA 311/1-2). J.D. gratefully acknowledges the support by the Bundesgesundheitsamt, Berlin, ZEBET (Grant Fo 2.1).

1. Bankey, P. E. & Cerra, F. B. (1993) in *Pathophysiology of Shock, Sepsis, and Organ Failure*, eds. Schlag, G. & Redl, H. (Springer, Berlin), pp. 948–957.
2. Renton, K. (1983) in *Biological Basis of Detoxication*, eds. Caldwell, J. & Jakoby, W. (Academic, New York), pp. 307–324.
3. Billiar, T. R., Curran, R. D., Stuehr, D. J., West, M. A., Bentz, B. G. & Simmons, R. L. (1989) *J. Exp. Med.* **169**, 1467–1472.
4. Curran, R. D., Billiar, T. R., Stuehr, D. J., Hofmann, K. & Simmons, R. L. (1989) *J. Exp. Med.* **170**, 1769–1774.
5. Curran, R. D., Billiar, T. R., Stuehr, D. J., Ochoa, J. B., Harbrecht, B. G., Flint, S. G. & Simmons, R. L. (1990) *Ann. Surg.* **212**, 462–469.
6. Geller, D. A., Nüssler, A. K., Di, S. M., Lowenstein, C. J., Shapiro, R. A., Wang, S. C., Simmons, R. L. & Billiar, T. R. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 522–526.
7. Nüssler, A., Drapier, J. C., Renia, L., Pied, S., Miltgen, F., Gentilini, M. & Mazier, D. (1991) *Eur. J. Immunol.* **21**, 227–230.
8. Geller, D. A., Lowenstein, C. J., Shapiro, R. A., Nüssler, A. K., Di Silvio, M., Wang, S. C., Nakayama, D. K., Simmons, R. L., Snyder, S. H. & Billiar, T. R. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 3491–3495.
9. Moncada, S., Palmer, R. M. & Higgs, E. A. (1991) *Pharmacol. Rev.* **43**, 109–142.
10. O'Keefe, D., Ebel, R. & Peterson, J. (1978) *J. Biol. Chem.* **253**, 3509–3516.
11. Wink, D. A., Osawa, Y., Darbyshire, J. F., Jones, C. R., Eshenaur, S. C. & Nims, R. W. (1993) *Arch. Biochem. Biophys.* **300**, 115–123.
12. Guengerich, F. P. (1987) *Mammalian Cytochromes P450* (CRC, Boca Raton, FL), Vols. 1 and 2.
13. Stadler, J., Billiar, T. R., Curran, R. D., Stuehr, D. J., Ochoa, J. B. & Simmons, R. L. (1991) *Am. J. Physiol.* **260**, C910–C916.
14. Morgan, E. T. (1989) *Mol. Pharmacol.* **36**, 699–707.
15. Barker, C., Fagan, J. & Pasco, D. (1992) *J. Biol. Chem.* **267**, 8050–8055.
16. Doehmer, J., Dogra, S., Friedberg, T., Monier, S., Adesnik, S., Glatt, H. & Oesch, F. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5769–5773.
17. Dogra, S., Doehmer, J., Glatt, H., Mölders, H., Siegert, P., Friedberg, T., Seidel, A. & Oesch, F. (1990) *Mol. Pharmacol.* **37**, 608–613.
18. Wölfel, C., Platt, K.-L., Dogra, S., Glatt, H., Wächter, F. & Doehmer, J. (1991) *Mol. Carcinog.* **4**, 489–498.
19. Schmalix, W., Mäser, H., Kiefer, F., Reen, R., Wiebel, F., Gonzales, F., Seidel, A. H. G., Greim, H. & Doehmer, J. (1993) *Eur. J. Pharmacol.* **248**, 251–262.
20. Wölfel, C., Heinrich-Hirsch, B., Schulz-Schalge, T., Seidel, A., Frank, H., Wächter, F., Wiebel, F. J., Gonzales, F., Greim, H. & Doehmer, J. (1992) *Eur. J. Pharmacol.* **228**, 95–102.
21. Seglen, P. O. (1976) *Methods Cell Biol.* **13**, 29–83.
22. Field, L., Dilts, R. V., Ravichandran, R., Lenhert, P. G. & Carnahan, G. E. (1978) *J. Chem. Soc. Chem. Commun.*, 249–250.
23. Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S. & Tannenbaum, S. R. (1982) *Anal. Biochem.* **126**, 131–138.
24. Nebert, D. & Gelboin, H. (1968) *J. Biol. Chem.* **243**, 6242–6249.
25. Wortelboer, H. M., De Kruif, C. A., Van Iersel, A. A. J., Falke, H. E., Noordhoek, J. & Blauboer, B. J. (1990) *Biochem. Pharmacol.* **40**, 2525–2534.
26. Laemmli, U. (1970) *Nature (London)* **227**, 680–685.
27. Burnette, W. N. (1981) *Anal. Biochem.* **112**, 195–203.
28. Chirgwin, J., Przybyla, A., MacDonald, R. & Rutter, W. (1979) *Biochemistry* **18**, 5294–5299.
29. Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254.
30. Curran, R. D., Ferrari, F. K., Kispert, P. H., Stadler, J., Stuehr, D. J., Simmons, R. L. & Billiar, T. R. (1991) *FASEB J.* **5**, 2085–2092.
31. Ignarro, L. J. (1990) *Pharmacol. Toxicol.* **67**, 1–7.
32. Stuehr, D. J. & Nathan, C. F. (1989) *J. Exp. Med.* **169**, 1543–1555.
33. Doehmer, J., Wölfel, C., Dogra, S., Doehmer, C., Seidel, A., Platt, K. L., Oesch, F. & Glatt, H. R. (1992) *Xenobiotica* **22**, 1093–1099.
34. Rao, D. N. R., Elguindi, S. & O'Brien, P. J. (1991) *Arch. Biochem. Biophys.* **286**, 30–37.
35. Kanner, J., Harel, S. & Granit, R. (1992) *Lipids* **27**, 46–49.
36. Stadler, J., Harbrecht, B. G., Di Silvio, M., Curran, R. D., Jordan, M. L., Simmons, R. L. & Billiar, T. R. (1993) *J. Leukocyte Biol.* **53**, 165–172.
37. Stadler, J., Bergonia, H. A., Di Silvio, M., Sweetland, M. A., Billiar, T. R., Simmons, R. L. & Lancaster, J. R. (1993) *Arch. Biochem. Biophys.* **302**, 4–11.
38. Fukuda, Y., Ishida, N., Noguchi, T., Kappas, A. & Sassa, S. (1992) *Biochem. Biophys. Res. Commun.* **184**, 960–965.
39. Nüssler, A. K., Di, S. M., Billiar, T. R., Hoffman, R. A., Geller, D. A., Selby, R., Madariaga, J. & Simmons, R. L. (1992) *J. Exp. Med.* **176**, 261–264.