Regulation of heme oxygenase-1 by nitric oxide during hepatopulmonary syndrome

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Carter, Ethan P., Cynthia L. Hartsfield, Motoaki Miyazono, Malathi Jakkula, Kenneth G. Morris, Jr., and Ivan F. McMurtry. Regulation of heme oxygenase-1 by nitric oxide during hepatopulmonary syndrome. Am J Physiol Lung Cell Mol Physiol 283: L346–L353, 2002.—During hepatopulmonary syndrome caused by liver cirrhosis, pulmonary endothelial nitric oxide (eNOS) expression and NO production are increased. Increased NO contributes to the blunted hypoxic pressor response (HPR) during cirrhosis and may induce heme oxygenase-1 (HO-1) expression and carbon monoxide (CO) production, exacerbating the blunted HPR. We hypothesized that NO regulates the expression of HO-1 during cirrhosis, contributing to hepatopulmonary syndrome. Cirrhosis was induced in rats by common bile duct ligation (CBDL). Rats were studied 2 and 5 wk after CBDL or sham surgery. Lung HO-1 expression was elevated 5 wk after CBDL. Liver HO-1 was increased at 2 wk and remained elevated at 5 wk. In catheterized rats, the blunted HPR was partially restored by NO inhibition. Rats treated with the NOS inhibitor Nω-nitro-L-arginine methyl ester for the entire 2- or 5-wk duration had normalized HO-1 expression and HPR. These data provide in vivo evidence for the NO-mediated upregulation of HO-1 expression and support the concept that hepatopulmonary syndrome is multifactorial, involving not only NO, but also HO-1 and CO.

carbon monoxide; cirrhosis; endothelial nitric oxide synthase; pulmonary vasoactivity; calcium-activated potassium channels

ENDOTHELIUM-DERIVED NITRIC OXIDE (NO) produced by the enzymatic activity of endothelial nitric oxide synthase (eNOS) has well-characterized actions as a vasodilator (33). Once produced, NO is freely diffusible and enters vascular smooth muscle cells (VSMCs) to activate soluble guanylate cyclase and produce guanosine 3′,5′-cyclic monophosphate (cGMP) (26, 33). In pulmonary artery (PA) VSMCs, increased cGMP activates a cGMP-sensitive kinase, which phosphorylates a calcium-dependent potassium (Kca) channel leading to hyperpolarization and vasodilation (7, 26, 33). Although most of the NO production in the vascular endothelium is due to eNOS, some studies suggest that the two other isoforms of NOS, inducible NOS and neuronal NOS, may also be present in the vasculature and contribute to NO production (20, 29, 30).

Heme oxygenase-1 (HO-1) catalyzes the rate-limiting step in the oxidative degradation of heme to biliverdin, releasing equimolar amounts of carbon monoxide (CO) and iron (5). Biliverdin is subsequently reduced to bilirubin by biliverdin reductase (5). CO, a gaseous messenger similar to NO, shares many properties with NO, including activation of guanylate cyclase, signal transduction, and gene regulation and may mediate important cellular functions (34).

In addition to its action as a vasodilator, NO can regulate the expression of a variety of genes. In particular, there is solid evidence that NO regulates the expression of HO-1 (1, 4, 15). For example, treating aortic smooth muscle cells with the NO donor spermine NONOate (SNN) increases HO-1 gene transcription, resulting in increased mRNA and protein expression (15). This induction of HO-1 by NO occurs in a cGMP-independent manner. The pathways by which NO regulates the expression of HO-1 and other genes are complex and not completely worked out but appear to involve mitogen-activated protein kinase (MAPK) members such as extracellular signal-regulated kinase (ERK) and p38 (4).

Often during liver cirrhosis there are pulmonary vascular complications leading to systemic hypoxemia (9, 10). This condition is termed the hepatopulmonary syndrome. A prominent feature of hepatopulmonary syndrome is a blunted hypoxic pressor response (HPR), that is, the pulmonary circulation has a reduced ability to vasoconstrict in the presence of hypoxic stimuli. Recent evidence has implicated NO in the pathogenesis of hepatopulmonary syndrome. Clinically, levels of exhaled NO are increased in cirrhotic patients (32). We (3) and others (11) have demonstrated that pulmonary expression of eNOS is increased during experimental cirrhosis induced in rats by common bile duct ligation (CBDL). However, we also have demonstrated that the vasodilatory action of NO alone does not completely account for the blunted HPR (3). We found that the acute inhibition of NOS and guanylate cyclase did not

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restore normal hypoxic vasoreactivity in cirrhotic rat lungs and that activation of PA smooth muscle cell Kca channels by both NO-dependent and -independent mechanisms caused hyperpolarization and vasodilation (3). In view of the evidence that NO can induce HO-1 expression (15) and that HO-1-derived CO can function as a vasodilator (3), we investigated the expression of HO-1 in two tissues central to the development of hepatopulmonary syndrome: lung and liver. There were two main objectives of this study: First, to determine whether HO-1 expression in the lung and liver is regulated in a tissue-specific fashion during CBDL-induced liver cirrhosis and whether there are functional consequences associated with such changes; second, to determine whether chronic inhibition of NO reverses the CBDL-induced changes in HO-1 expression and restores normal hypoxic pulmonary vasoreactivity. We hypothesized that HO-1 expression in liver would be upregulated early, closely followed by increased expression in lung. We further hypothesized that upregulation of HO-1 could be blocked by chronic inhibition of NOS. Our major findings were that after CBDL, hepatic HO-1 was potently upregulated, closely followed by pulmonary HO-1. Inhibition of HO-1 activity partially restored the blunted HPR in CBDL rats, functionally implicating HO-1 in the blunted HPR. Finally, both the HO-1 induction and blunted HPR were abolished by chronic N^N^-nitro-L-arginine methyl ester (L-NAME) treatment.

METHODS

Animal model of liver cirrhosis. Biliary cirrhosis was induced in rats by CBDL (3, 12, 22). The surgical procedures were approved by the Animal Care and Use Committee at the University of Colorado Health Sciences Center. Male Sprague-Dawley rats [200–250 g body wt (bw)] were allowed to acclimate to Denver’s altitude (1,500 m) for 1 wk before any experimental protocols. Animals had continuous access to food and water. Laparotomy was performed under anesthesia of ketamine (100 mg/kg im) and xylazine (4 mg/kg im). The bile duct was isolated, doubly ligated with 3-0 silk, and resected between the two ligatures. The abdominal wall was closed with 4-0 silk, antibiotic sulfathiazole powder sprinkled over the closure, and the skin was closed with 4-0 silk. Buprenorphine (0.25 mg/kg) was given subcutaneously twice during the first 24 h after surgery to alleviate postsurgical discomfort. Sham animals underwent laparotomy, bile duct isolation, no ligation and resection, and closure of the surgical opening. Experiments were carried out 2 or 5 wk after surgery. We evaluated liver injury by measuring serum levels of bilirubin using a bilirubin direct assay (Sigma, St. Louis, MO).

Chronic inhibition of NOS. One day after surgery, specified groups of sham and CBDL rats received the nonselective NOS inhibitor L-NAME in their drinking water (6 mg/l, ~0.072 mg·day^-1·100 g bw^-1). This dose in rats normalizes the systemic vascular changes that occur during cirrhosis (24) and pregnancy (2) without causing systemic hypertension. Rats received the L-NAME treatment for the duration of the 2- or 5-wk experiment.

HO activity inhibition. To investigate the role of HO and HO-derived CO in the blunted HPR, we treated specified groups of sham and CBDL rats with the specific HO inhibitor zinc protoporphyrin (ZnP, 50 μmol/kg ip). Rats were treated once, 18 h before we measured hemodynamics described in the next section. During and after ZnP treatment, rats were kept in low-light conditions because of the high photoinactivation of porphyrin compounds.

Arterial blood gas and hemodynamic measurements. At 2 and 5 wk postsurgery, rats were anesthetized with ketamine and xylazine, as described in Animal model of liver cirrhosis. Catheters (PE-50 tubing) were placed in the carotid artery and PA via the right jugular vein and right ventricle. The catheters were passed subcutaneously and exteriorized at the scapulae. Rats were allowed to recover for 24 h before arterial blood gases, mean arterial pressure (MAP), and pulmonary artery pressure (PAP) were measured in conscious rats breathing room air, 10% O2, and 100% O2. The change in PAP from room air to 10% O2 was defined as the HPR. In addition, blood carboxyhemoglobin (COHb) was used as an index for the amount of CO in the blood. Arterial blood (30–50 μl) was collected in a heparinized syringe at the beginning of studies. Samples were measured by an OMS3 Hemoxymeter (Radiometer, Copenhagen, Denmark).

Northern blot analysis of lung and liver HO-1 mRNA expression. Rats were anesthetized with ketamine and xylazine, and the PA and left ventricles were perfused with PBS containing heparin to flush free of blood the lungs and systemic organs, respectively. The left lung and one liver lobe were excised and immediately placed in 8 ml of homogenizing solution (4 M guanidine, 0.1 M Tris, pH 7.5, 0.1 M 2-mercaptoethanol) and homogenized for 45 s with a torque grinder. After homogenization, 50 μl of 10% sarkosyl/ml homogenate (final concentration = 0.5% sarkosyl) were added, and the tubes were frozen in liquid nitrogen. The right lung and a second liver lobe were removed and processed for protein extraction, as described below. Total RNA was extracted from peripheral lung and liver by a standard guandinium-phenol-chloroform extraction protocol. We determined the quantity of RNA by measuring the optical density at 260 nm (OD260 = 1 for 40 μg/ml RNA), and we assessed the purity by determining the ratio of the optical density obtained 260 and 280 nm (pure RNA: OD260/OD280 = 2.0) using a Beckman DU-640 spectrophotometer. Total RNA (20 μg) was electrophoresed through a formaldehyde-agarose gel and blotted to a nylon membrane using capillary transfer. RNA was immobilized on the nylon membrane by ultraviolet cross-linking. HO-1 mRNA was detected with a 2.0-kb cDNA probe labeled with [α-32P]dCTP using random-primed labeling (RTS Random Primer DNA Labeling System; GIBCO-BRL, Gaithersburg, MD). 18S rRNA levels were measured by hybridization with an oligonucleotide probe (ACCGTATCTGATCCGTCGCTGCCAGAAC) labeled with [α-32P]dCTP using terminal deoxynucleotidyl transferase. After hybridization, blots were washed at room temperature in 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0 (1× SSC)-0.1% SDS (low stringency) and then at 65°C in 0.4× SSC-0.1% SDS (high stringency). We obtained autoradiographs by exposing blots to phosphorimaging cassettes, and densitometry was performed on a phosphorimaging system with ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Western blot analysis of HO-1 expression. As with the Northern blot analysis, standard techniques were used to evaluate protein expression. The right lung and one liver lobe, from the above blood-free perfusion, were homogenized in 250 mM sucrose, 25 mM imidazole, 1 mM EDTA, and 1/10 volume of a protease solution consisting of 25 μg/ml antipain, 1 μg/ml aprotinin, 0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin, 0.1 mg/ml soybean trypsin inhibitor, and 200 μM phenylmethylsulfonyl fluoride. After homogenization, samples were...
sonicated and spun at low speed to clear debris, and the supernatant was frozen at −80°C until assayed for protein content. SDS-PAGE/immunoblotting was performed on 25 μg of protein. Samples were electrophoresed through a 12% acrylamide gel for detection of HO-1 (molecular mass = 30 kDa). HO-1 protein was detected using a polyclonal antibody (StressGen Biotechnologies, Victoria, British Columbia, Canada) diluted 1:1,000 in Tris-buffered saline + 0.1% Tween 20 (TBS-T) containing 5% dry milk. The secondary antibody (sheep anti-rabbit conjugated to horseradish peroxidase) was diluted 1:17,000 in TBS-T containing 5% dry milk. Antigenic detection was by enhanced chemiluminescence (Amersham, Arlington Heights, IL) with exposure to X-ray film. Densitometry was performed with a scanner and NIH Image software (version 1.61).

Statistical analysis. All values reported are means ± SE. We carried out comparisons between groups using the analysis of variance followed by Tukey’s post hoc analysis. Values of P < 0.05 were considered significant. Sample sizes of specific groups are listed in the figure legends (Figs. 1–6).

RESULTS

Characterization of cirrhosis induction by CBDL. The physiological and biochemical changes associated with CBDL have been previously reported (3, 12, 22). As is typical after CBDL, serum levels of bilirubin were elevated by 2 wk after CBDL, indicative of liver injury (Table 1). CBDL rats were slightly hypoxicemic by 2 wk and more profoundly so by 5 wk (Table 1). However, when the rats breathed 100% O2, the differences in arterial O2 tension between the sham and CBDL rats were no longer present. MAP was slightly lower in the 2-wk CBDL rats and significantly lower in the 5-wk CBDL rats compared with time-matched sham rats (Table 1).

HO-1 expression is increased after CBDL. We and others have previously found that after CBDL, lung eNOS protein expression is increased (3, 11) and preproendothelin-1 mRNA is decreased (3). We compared the pattern of expression of HO-1 in the lung and liver to determine whether the expression resembles that of eNOS. In the lung, the increased HO-1 protein expression in lung followed a similar pattern of expression as with the mRNA, with a slight increase at 2 wk and a greater than fivefold increase by 5 wk (Fig. 1B). In the liver, HO-1 mRNA induction was more rapid and sustained, with a significant 14-fold induction at 2 wk and nearly fivefold 5 wk after CBDL compared with time-matched controls (Fig. 2A). Similarly, hepatic HO-1 protein expression in CBDL rats was elevated nearly fivefold at 2 wk and threefold by 5 wk (Fig. 2B).

Circulating CO is increased after CBDL. A byproduct of HO-1 activity is CO. HO-1 activity is the primary source of circulating CO (17). In the circulation, CO is found predominately bound to hemoglobin in the form of COHb. To determine whether elevated levels of circulating CO accompanied the strong induction of HO-1 in the lung and liver, we measured the amount of COHb in the blood of sham and CBDL rats. Figure 3 illustrates that levels of COHb were significantly elevated at both the 2-wk and 5-wk time points in the blood from CBDL rats compared with sham rats.

HO activity contributes to blunted HPR. To evaluate the functional consequences of the HO-1 induction, we treated sham and CBDL rats with the specific HO inhibitor ZnPP. Studies were carried out at the 5-wk time point when HO-1 induction and HPR blunting were maximal. HO inhibition resulted in partial restoration of the blunted HPR in the CBDL rats (Figure 4). ZnPP treatment altered neither the HPR in sham rats (Fig. 4) nor the systemic pressure in sham and CBDL rats (not shown).

NOS inhibition blocks HO-1 induction and lowers COHb levels in CBDL rats. Elevated NO has been implicated in the pulmonary and systemic circulatory abnormalities during cirrhosis. To determine what effect NOS inhibition has on cardiovascular dynamics and HO-1 expression during cirrhosis, we treated sham and CBDL rats with L-NAME for either 2 or 5 wk. The dose of L-NAME used did not cause systemic hypertension in sham rats and reversed the systemic hypotension in the CBDL rats (Table 1). L-NAME treatment abolished the hypoxemia in CBDL rats during room air breathing. This was associated with restoration of the HPR, which was not different between the sham and CBDL rats treated with L-NAME (Fig. 5).

To determine whether elevated NO that occurs after CBDL was responsible for the induction of pulmonary and hepatic HO-1, we examined HO-1 expression in rats treated with L-NAME for 2 and 5 wk after CBDL. In the lung, the increased HO-1 protein expression after CBDL was prevented and reduced, respectively, at the 2- and 5-wk time points, respectively (Fig. 6A). Of the eight CBDL lungs studied at the 5-wk time point, rats treated with L-NAME had nearly 14-fold induction at 2 wk and threefold at 5 wk (Fig. 6B).

Table 1. Arterial oxygenation, mean arterial pressure, and serum bilirubin after common bile duct ligation

<table>
<thead>
<tr>
<th></th>
<th>Sham 2-Wk</th>
<th>CBDL 2-Wk</th>
<th>Sham 5-Wk</th>
<th>CBDL 5-Wk</th>
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<tbody>
<tr>
<td>Pao2 (room air), mmHg</td>
<td>79.7 ± 0.9</td>
<td>73.9 ± 0.7*</td>
<td>80.1 ± 0.7</td>
<td>70.5 ± 0.9*</td>
</tr>
<tr>
<td>Pao2 (100% O2), mmHg</td>
<td>468 ± 13</td>
<td>459 ± 11</td>
<td>469 ± 19</td>
<td>478 ± 17</td>
</tr>
<tr>
<td>Pao2 after L-NAME (room air), mmHg</td>
<td>78.5 ± 2.5</td>
<td>77.6 ± 1.0</td>
<td>80.6 ± 0.3</td>
<td>77.9 ± 1.1</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>125.8 ± 1.7</td>
<td>118.3 ± 8.1</td>
<td>124.3 ± 2.7</td>
<td>105.1 ± 3.2†</td>
</tr>
<tr>
<td>MAP after L-NAME, mmHg</td>
<td>127.8 ± 4.2</td>
<td>113.7 ± 3.9</td>
<td>125.6 ± 3.4</td>
<td>120.9 ± 4.9</td>
</tr>
<tr>
<td>Serum bilirubin, mg/dl</td>
<td>0.10 ± 0.01</td>
<td>11.31 ± 1.67†</td>
<td>0.11 ± 0.02</td>
<td>12.47 ± 1.97†</td>
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Values are means ± SE. *Different from time-matched sham, P < 0.001. † Different from time-matched sham, P < 0.05, n = 5–8 per group.

CBDL, common bile duct ligation; L-NAME, Nω-nitro-L-arginine methyl ester; MAP, mean systemic arterial pressure.
point, L-NAME treatment completely reversed the HO-1 induction in four and partially inhibited the L-NAME induction in the other four. In the liver, the strong induction by CBDL was prevented by L-NAME treatment in all of the rats studied (Fig. 6A). Densitometric analysis of lung and liver HO-1 protein expression from the entire set of L-NAME-treated rats is shown (Fig. 6B). Consistent with the reduced HO-1 expression in CBDL rats after L-NAME treatment, the level of circulating COHb was also significantly reduced (Fig. 3).

DISCUSSION

We have recently reported that eNOS expression in lung and plasma NO metabolites (NOx) levels are elevated after CBDL but that the acute inhibition of NO only partially reversed the blunted hypoxic pulmonary vasoconstriction (3). We therefore considered the possibility that the elevated NO during cirrhosis was acting not only as a vasodilator, but also as a regulator of gene expression of other vasoactive mediators such as HO-1 (8, 14). CO, an HO-1 byproduct, activates guanylate cyclase, leading to accumulation of cGMP and VSMC relaxation (6, 13, 16, 18, 23, 31). CO can also cause vasodilation by cGMP-independent pathways, possibly by directly activating KCa channels (19, 35–37) and/or inhibiting production of endothelin (25). Thus we postulated that pulmonary HO-1 is induced by NO and that HO-1-derived CO could be contributing to the NO-independent, KCa channel-dependent vasodilation we have recently reported (3). Therefore, there were two main objectives of this study: First, to deter-

**Fig. 1.** Northern and Western blot analysis of heme oxygenase (HO)-1 expression in lung from sham and common bile duct ligated (CBDL) rats. A: 25 μg of lung total RNA were loaded per lane, and the blot was probed for HO-1 mRNA (top) or 18s rRNA (middle). HO-1 mRNA expression relative to 18s rRNA was quantitated (bottom). B: 25 μg of lung protein homogenate were loaded per lane. The blot was probed as described in METHODS and exposed to film (top). Densitometry was done to quantify HO-1 protein expression (bottom). The blot is representative of 2 blots with a total of 5–8 samples/group between the 2 blots. *Significantly greater than sham data from same time point, \( P < 0.05 \).
mine whether HO-1 expression in lung and liver is regulated in a tissue-specific fashion during CBDL-induced liver cirrhosis and whether there are functional consequences associated with such changes; second, to determine whether chronic inhibition of NO reverses the CBDL-induced changes in HO-1 expression and restores normal hypoxic pulmonary vasoreactivity.

We observed that after CBDL, HO-1 expression in the lung closely followed the pattern of expression of eNOS previously observed (3, 11). Regulation of HO-1 expression by NO has been demonstrated in a variety of cell types, primarily in cell culture experiments (1, 8, 15). VSMCs treated with the NO donor SNN for 4 h had a 105-fold induction of HO-1 mRNA (15). Treatment of rat aortic VSMCs with the NO donors sodium nitroprusside (SNP), S-nitroso-N-acetyl-penicillamine, or 3-morpholinosydnonimine increases HO-1 mRNA and protein expression in a concentration- and time-dependent manner (8). In HeLa cells treated with SNP, the NO-dependent HO-1 induction took place via MAPK ERK (4). During cirrhosis, it has been widely reported that circulating levels of NOX (3), pulmonary expression of eNOS (3, 11, 27), and exhaled amounts of NO (32) are all elevated. Our data, together with these earlier reports, support our hypothesis that NO regulates HO-1 expression in our in vivo model similarly to what has been reported in in vitro models.

Fig. 2. Northern and Western blot analysis of HO-1 expression in liver from sham and CBDL rats. A: 25 μg of liver total RNA were loaded per lane, and the blot was probed for HO-1 mRNA (top) and 18s rRNA (middle). HO-1 mRNA expression relative to 18s rRNA was quantitated (bottom). B: 25 μg of liver protein homogenate were loaded per lane. The blot was probed as described in METHODS and exposed to film (top). Densitometry was done to quantitate HO-1 protein expression (bottom). The blot is representative of 2 blots with a total of 5–8 samples/group between the 2 blots. *Significantly greater than time-matched sham data, P < 0.05.
We hypothesized that NO regulates the expression of HO-1 during cirrhosis. This hypothesis was tested through the chronic inhibition of NOS activity by L-NAME added to the rats’ drinking water for 2 or 5 wk. A common occurrence of NOS inhibition by L-NAME and similar compounds is the development of systemic hypertension. We selected a dose of L-NAME that has previously been shown to inhibit NOS activity without causing systemic hypertension (2, 24). In our experiments, MAP was not different between the L-NAME-treated and untreated rats (Table 1). In CBDL rats treated with L-NAME, the induction of HO-1 was markedly reduced in both the lung and liver. Although these results support our hypothesis of NO being a regulator of gene expression during cirrhosis, we acknowledge that HO-1 gene expression is certainly more complex than NO’s simply acting as an enhancer of HO-1 transcription. We, therefore, have to consider that additional factors may be regulating HO-1 expression. These may include flow and shear stress phenomena and growth factors and cytokines.

In addition to the normalization of HO-1 expression, chronic L-NAME treatment also preserved hypoxic pulmonary vasoconstriction in CBDL rats. This is an important physiological observation linking both NO and HO-1 to the blunted HPR during hepatopulmonary syndrome. Although the beneficial effects of NO inhibition on renal function during cirrhosis have been reported (24), our data along with those recently reported by Nunes et al. (28) are the first to demonstrate that chronic inhibition of NO production may ameliorate hepatopulmonary syndrome.

Although HO-1 expression was elevated in both the lung and liver of CBDL rats, the time course and magnitude of induction were different between the two organs. In the liver, HO-1 mRNA induction was rapid, sustained, and of a greater magnitude than that in the lung. The rapid onset of HO-1 induction at the 2-wk time point may be due to the fact that the injury originates in the liver and HO-1 induction is a stress response to the local hepatic injury during CBDL. The greater magnitude of HO-1 mRNA induction compared with that in the lung might be due to the fact that HO-1 is responding to both elevated NO and the continual stress response created by CBDL injury. Because the HO-1 mRNA induction in lung was slower (not until wk 5) and of a lesser magnitude (2-fold vs. 14-fold) than in liver, it is possible that local stress-response factors from the liver are not governing HO-1 expression in the lung. Rather, elevated lung NO may be the primary mechanism by which lung HO-1 expression is increased during cirrhosis. A larger elusive question still remains of what factor(s) is behind the induction of eNOS and NO during cirrhosis both sys-
Fig. 5. Pulmonary HPR measured in vivo in sham and CBDL rats chronically treated with L-NAME at 2 wk (A) and 5 wk (B). Pulmonary artery pressure (PAP) was measured in awake, catheterized rats breathing room air (21% O₂) and hypoxic air (10% O₂). The increase in PAP during 10% O₂ breathing is defined as the HPR. Note the blunted HPR in the CBDL rats not treated with L-NAME and the potentiation of the HPR after chronic L-NAME treatment. n = 4–6 rats/group. Values are means ± SE. *Significantly greater than room air measurements from same group, P < 0.05.

Fig. 6. Western blot analysis of HO-1 expression in lung and liver from sham and CBDL rats chronically treated with L-NAME. A: 25 μg of lung (top) and liver (bottom) protein homogenate were loaded per lane and electrophoresed. In the lung blot (top), a control sample (C) marked of lung tissue from non-L-NAME-treated rats was electrophoresed. The blots were probed as described in METHODS and exposed to film. B: the results from densitometric analyses are shown for lung (left) and liver (right). A total of 7–8 animals were studied for each time point.
contributes to the blunted HPR and 2) as a regulator of expression of other vasoactive genes, such as HO-1.

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