Induction of Heme Oxygenase-1 Expression in Vascular Smooth Muscle Cells

A LINK TO ENDOTOXIC SHOCK

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Endotoxic shock is a life-threatening consequence of severe Gram-negative infection characterized by vascular smooth muscle cell relaxation and severe hypotension. The production of nitric oxide (NO), through the inducible NO synthase pathway, has been implicated as a major contributor in this process. We now demonstrate that heme oxygenase (HO), an enzyme that generates carbon monoxide (CO) in the course of heme metabolism, may also be involved in the hemodynamic compromise of endotoxic shock. Inducible HO (HO-1) mRNA levels are dramatically increased in aorti tissue from rats receiving endotoxin, and this increase in vascular HO-1 message is associated with an 8.9-fold increase in HO enzyme activity in vivo. Immunocytochemical staining localizes an increase in HO-1 protein within smooth muscle cells of both large (aorta) and small (arterioles) blood vessels. Furthermore, zinc protoporphyrin IX, an inhibitor of HO activity, abrogates endotoxin-induced hypotension in rats. Studies performed in rat vascular smooth muscle cells in vitro show that the induction of HO-1 mRNA is regulated at the level of gene transcription, and this induction is independent of NO production. Taken together, these studies suggest that the up-regulation of HO-1, and the subsequent production of CO, contributes to the reduction in vascular tone during endotoxic shock.

Endotoxemia leading to shock is a detrimental consequence of severe Gram-negative bacterial infection. Endotoxic shock is initiated by the release of bacterial cell wall-derived lipopolysaccharide (LPS) and the subsequent production of cytokines and vasoactive mediators that result in vascular smooth muscle cell relaxation and hypotension. One of the most important cytokines in the cascade of events leading to LPS-induced hypotension is interleukin (IL)-1β. We have demonstrated previously that IL-1β stimulates the inducible isform of nitric oxide synthase (NOS) and increases the production of NO in vascular smooth muscle cells. NO is a labile, free radical gas that acts as a potent vasodilator. The importance of NO in the pathogenesis of endotoxic shock has been emphasized by recent studies demonstrating that mice carrying a disrupted inducible NOS gene have an attenuated hypotensive-response to LPS and are resistant to LPS-induced death. However, the study by MacMicking and colleagues also suggested that an inducible NOS-independent pathway contributes to LPS-induced hypotension and death, and we hypothesize that one potential pathway involves heme oxygenase (HO).

HO is the enzyme that generates carbon monoxide (CO) and biliverdin (subsequently reduced to bilirubin) in the course of heme metabolism. CO is a gas molecule that shares some of the properties of NO, inasmuch as CO binds to the heme moiety of cytosolic guanylyl cyclase to produce cGMP. Two distinct forms of heme oxygenase have been identified: HO-1 (an inducible isozyme) and HO-2 (a non-inducible isozyme). Morita and colleagues have demonstrated that HO-1 is induced by hypoxia in vascular smooth muscle cells in vitro, and that its product, CO, promotes the accumulation of cGMP in this cell type. The investigators also showed that smooth muscle cell-derived CO inhibits the production of endothelium-derived vasoactive agents (such as endothelin-1 and platelet-derived growth factor-B) under hypoxic conditions. Furthermore, a recent study by Raju and Maines demonstrated that expression of HO-1 in the cardiovascular system is up-regulated in vivo using a model of acute renal ischemia and reperfusion. The authors speculated that increased HO-1 expression, and the ensuing CO production, may promote vasodilation as a defense response to renal ischemia/reperfusion. These previous studies (both in vitro and in vivo) suggest a physiologic role for CO in vascular biology.

We designed the present study to further understand the role of CO in endotoxic shock by 1) analyzing the regulation of vascular HO-1 in vivo, 2) administering zinc protoporphyrin IX (ZnPP), an inhibitor of HO activity, to rats made hypotensive by LPS, and 3) investigating the mechanism of HO-1 induction in vascular smooth muscle cells in vitro.

**EXPERIMENTAL PROCEDURES**

*Materials—Salmonella typhosa LPS (Sigma) was dissolved in 0.9% saline and stored at −20 °C. Recombinant human IL-1β (Collaborative Biomedical, Bedford, MA) was stored at −80 °C until use. ZnPP (Porphyrin Products, Logan, UT) was dissolved in 0.1 N NaOH, then immediately prior to administration this solution was neutralized with 0.1 N HCl as described (16). Because ZnPP has effects unrelated to HO inhibition when exposed to light (15), we protected the ZnPP from light during its preparation and use. Both the t-arginine analogue N₆-nitro-t-arginine methyl ester (t-NAME; Calbiochem, San Diego, CA) and S-methylisothiourea sulfate (SMT; Sigma) were dissolved in sterile*
water and stored at -20 °C until their use.

**Cell Culture**—Rat aortic smooth muscle cells (RASMCS) were harvested from male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) by enzymatic dissociation according to the method of Gunther et al. (18). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum (FBS) and supplemented with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and 25 mM Hepes (pH 7.4) (Sigma). RASMCS were passaged every 4–7 days, and experiments were performed on cells 6–8 passages from primary culture. After the cells had grown to confluence, they were placed in 2% fetal calf serum 12 h before the experiments.

**RNA Blot Hybridization**—Total RNA was obtained from rat aortas and cultured smooth muscle cells by guanidinium isothiocyanate extraction and centrifuged through cesium chloride (17). The RNA was fractionated on a 1.3% formaldehyde-agarose gel and transferred to a nitrocellulose filter. The filters were hybridized at 68 °C for 2 h with 32P-labeled rat HO-1 or HO-2 probes (11) in QuikHyb solution (Stratagene, La Jolla, CA). The hybridized filters were then washed in 30 mm sodium chloride, 3 mm sodium citrate, and 0.1% sodium dodecyl sulfate solution at 55 °C and autoradiographed with Kodak XAR film at -80 °C for 12 h or stored on phosphor screens for 2–4 h. To correct for differences in RNA loading, the filters were washed in a 50% formamide solution at 80 °C and rebound to an 18 S ribosomal RNA probe. Images were displayed, and radioactivity was measured on a PhosphorImager running ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

**Nuclear Run-on Analysis**—RASMCS were either not stimulated (vehicle) or stimulated with IL-1β (10 ng/ml) for 24 h. The cells were subsequently lysed, and nuclei were isolated as described (4, 18). Nuclear suspension (200 μl) was incubated with 0.5 μl each of CTP, ATP, and GTP and with 250 μCi of 32P-labeled UTP (3,000 Ci/mmol) (Du Pont NEN). The samples were extracted with phenol/chloroform, precipitated, and resuspended at equal counts/min/ml in hybridization buffer (9.7 × 106 cpm/ml). Denatured probes (1 μg) dot-blotted on nitrocellulose filters were hybridized at 40 °C for 4 days in the presence of formamide. cDNA for HO-1, HO-2, and β-actin genes were used as probes.

**HO Enzyme Activity**—Aortas were harvested from male Sprague-Dawley rats (200–250 g) treated with vehicle or LPS (4 mg/kg intravenously), the adventitia was stripped, and the tissue was homogenized with Polytron in homogenization buffer (30 mm Tris, pH 7.5, 0.25 mM sucrose, 0.15 mM NaCl) containing Complete protease inhibitor (Boehringer Mannheim). The homogenate was centrifuged at 10,000 x g for 1 h. The microsomal pellet was resuspended in 50 mM potassium phosphate buffer (pH 7.4) containing Complete™ protease inhibitor (Boehringer). The homogenate was centrifuged at 10,000 x g for 1 h. The microsomal supernatant fraction from the control animal served as the source of biliverdin reductase. A reaction mixture (0.5 ml) containing 33 nmol of bilirubin, 10 μl of microsomal supernatant fraction, NADPH generating system, and aortic microsomal protein was incubated at 37 °C for 10 min in the dark. The reaction mixture without NADPH generating system served as a blank. The reactions were stopped by placement on ice, and subsequently scanned with a spectrophotometer (Beckman, Columbia, MD). The amount of bilirubin formed was determined as the difference in optical density units between 426 and 530 nm (extraction coefficient, 40 nmol cm⁻¹ for bilirubin). HO enzyme activity was expressed as nanomoles (nmol) of bilirubin formed/mg of protein/h. The protein concentration was determined by a dye-binding assay (Bio-Rad).

**Immunocytochemical Staining**—Male Sprague-Dawley rats (200–250 g) treated with LPS or vehicle were perfused with 4% paraformaldehyde. The aortas were removed, post-fixed with 4% paraformaldehyde overnight at 4 °C, and then soaked in 30% sucrose for 2 days at 4 °C. The specimens were cut at a thickness of 5 μm. Immunocytochemical procedure was performed as described (21, 22). To reduce nonspecific binding, the sections were incubated in phosphate-buffered saline containing 10% normal goat serum and 0.4% Triton X-100 for 30 min. Rabbit polyclonal antibody against purified rat liver HO-1 (StressGen Biotechnologies, Victoria, BC, Canada) was applied for 1 h at room temperature and then overnight at 4 °C at a dilution of 1:1000–1200. Sections were washed with PBS several times with 0.5 μM NaCl and once with regular phosphate-buffered saline (5 min for each wash) and then incubated with biotinylated goat anti-rabbit IgG at a dilution of 1:500 for 1 h at room temperature. They were then rinsed with phosphate-buffered saline and incubated with avidin-biotin complex (ABC elite kit, Vector Laboratories, Burlingame, CA) at a dilution of 1:100 for 1 h at room temperature. After washing with PBS, the sections were treated with diaminobenzidine in phosphate buffered saline-H2O2 for 1–3 min using the peroxidase substrate kit DAB (Vector) and then transferred into phosphate buffered saline solution to stop the reaction. The presence of HO-1 was indicated by the development of a brown color within the cytoplasm. Counterstaining was performed with 0.5% methyl green.

**Nitrite Assay**—To determine the amount of NO produced by RASMCS, we measured a stable product of NO oxidation, NO2 −, by a nitrite assay. Male Sprague-Dawley rats (200–250 g) treated with LPS (4 mg/kg intravenously) or vehicle were perfused with 4% paraformaldehyde overnight at 4 °C and then soaked in 30% sucrose for 2 days at 4 °C. The nervous system was cut into 50 μm thick sections. The tissue sections were treated with diaminobenzidine in phosphate buffered saline-H2O2 for 1–3 min using the peroxidase substrate kit DAB (Vector) and then transferred into phosphate buffered saline solution to stop the reaction. The presence of HO-1 was indicated by the development of a brown color within the cytoplasm. Counterstaining was performed with 0.5% methyl green.

**Hemodynamic Experiment**—Male Sprague-Dawley rats (Charles River Laboratories) weighing 175–200 g received an intraperitoneal injection of thiobutabarbital sodium (100 mg/kg), which kept them anesthetized throughout the experiment. The trachea was cannulated with a tubing adapter. Then the right carotid artery was cannulated with PE-50 tubing to measure arterial pressure (24, 25) using MacLab monitoring equipment from ADInstruments, Inc. (Milford, MA). Saline (0.9%) was used as a control for LPS and ZnPP. The LPS group received LPS (4 mg/kg intra-arterially) and saline vehicle in place of ZnPP. The LPS + ZnPP groups received LPS (4 mg/kg intra-arterially) followed by ZnPP (10 μmol/kg or 1 μmol/kg intraperitoneally). The ZnPP group received ZnPP (10 μmol/kg intraperitoneally) and saline vehicle in place of LPS. After an initial 25% decrease in mean arterial pressure (corresponding to time point 0) in the rats receiving LPS (LPS group and LPS + ZnPP groups), ZnPP (or saline vehicle) was administered and mean arterial pressure was monitored over the next 90 min. Preliminary experiments revealed that all rats responded to LPS with time; however, if a rat’s mean arterial pressure did not decrease within 2 h, it was excluded from the study. Thus, only the rats most sensitive to LPS were used and it was not necessary to prolong their time under anesthesia.

**Data Analysis**—Comparisons between the vehicle and LPS-treated groups for HO enzyme activity were made using unpaired t tests (two-tailed). Comparisons of hemodynamic data between groups were made using analysis of variance (ANOVA) with Duncan’s multiple range test.
by factorial analysis of variance followed by Fisher’s least significant difference test, or unpaired t tests. Statistical significance was accepted for a p value < 0.05.

RESULTS

**LPS Induces Vascular HO-1 mRNA and Enzyme Activity in Vivo**—To determine if LPS regulates vascular HO-1 in an animal model of endotoxic shock, we injected rats with vehicle or *S. typhosa* LPS (4 mg/kg intravenously). HO-1 mRNA levels were markedly increased in aortic tissue after 9 h of LPS stimulation compared with tissue from rats receiving vehicle (Fig. 1A). We have demonstrated previously that this dose of LPS produces hypotension in rats (25), and the 9-h time point was chosen after performing an in vivo time-course experiment to assess maximal HO-1 mRNA induction. Moreover, LPS induced HO-1 message as early as 2 h after stimulation, and LPS did not increase HO-2 mRNA levels (data not shown).

We next assessed whether the increase in HO-1 mRNA levels corresponded to an increase in HO enzyme activity. Rats were given vehicle or LPS (4 mg/kg intravenously), and the aortas were harvested 9 h later (adventitia of the vessels was stripped prior to analysis). LPS promoted an 8.9-fold increase \( p < 0.05 \) in HO enzyme activity (Fig. 1B). In fact, the level of HO enzyme activity in the aortic tissue from rats receiving LPS (36.3 ± 2.4 nmol/mg of protein/h) was comparable to the level of HO activity in the liver of control rats (40 ± 2.1 nmol/mg of protein/h, \( p = 0.34 \)). The activity of HO is typically highest in organs rich in reticuloendothelial cells (i.e., liver, spleen, and bone marrow) (9, 10). These data demonstrate the significant amount of inducible HO enzyme activity generated in vascular tissue after LPS stimulation. Preliminary experiments were also performed to determine a dose of ZnPP that would suppress, but not abolish, vascular HO activity in vivo. When ZnPP was administered at a dose of 10 μmol/kg (intraperitoneally) 1 h after LPS, the level of vascular HO enzyme activity (6.1 nmol/mg of protein/h) was very similar to that of control rats (Fig. 1B). Thus, ZnPP doses of 10 and 1 μmol/kg were used in subsequent hemodynamic experiments.

To localize the cell type within the vessel responsible for the increase in HO enzyme activity, immunocytochemical staining was performed using a rabbit anti-HO-1 antibody. Staining for HO-1 protein was increased in the smooth muscle cells of aortas from rats receiving LPS (Fig. 2A) compared with rats receiving vehicle (Fig. 2A). Moreover, immunocytochemical staining demonstrated an LPS-induced increase in HO-1 expression in the smooth muscle cells of arterioles (Fig. 2, E and F compared with C and D), smaller vessels that contribute to the regulation of vascular tone. Staining for HO-1 was also increased in the endothelium of aortas and arterioles after LPS stimulation. These data demonstrate an increase in vascular HO-1 mRNA and protein levels after LPS administration in vivo and, more importantly, an increase in HO enzyme activity.

**ZnPP Abrogates the Hypotension Produced by LPS**—To provide evidence that HO contributes to the reduction in arterial pressure associated with endotoxemia, we gave ZnPP to rats made hypertensive by LPS. *S. typhosa* LPS given at a dose of 4 mg/kg intra-arterially produced profound and reproducible hypotension in male Sprague-Dawley rats (LPS group, Fig. 3). We then gave the same dose of LPS to another group of rats, followed by ZnPP (10 or 1 μmol/kg intraperitoneally) after an initial 25% decrease in mean arterial pressure (LPS+ZnPP groups, Fig. 3). By the time the arterial pressure had decreased by 25%, HO-1 mRNA levels had increased by 4-fold (Fig. 3, inset). ZnPP given at a dose of 10 μmol/kg after the onset of LPS-induced shock abolished the hypotension and produced a significantly higher mean arterial pressure than the LPS group after 30, 60, and 90 min \( p < 0.05 \). ZnPP administered at a dose of 1 μmol/kg arrested the LPS-induced hypotension, but the response was significantly less than in the higher dose (10 μmol/kg). LPS+ZnPP group after 30 and 60 min \( p < 0.05 \). ZnPP did not significantly increase mean arterial pressure when given at the dose of 10 μmol/kg intraperitoneally to rats not receiving LPS (ZnPP group, Fig. 3). The lack of an increase in arterial pressure again suggests this dose of ZnPP (10 μmol/
arterial pressure in LPS and LPS
10 aortic tissue of rats at base-line level or after LPS stimulation corre-
4298 tion (within 2 h), IL-1
24 h. The time course of HO-1 mRNA stimulation by IL-1
b increases HO-1, but Not HO-2, mRNA in Vascular Smooth Muscle Cells in Vitro—To further understand the molecu-
lar mechanisms regulating vascular HO-1, we investigated the induction of HO-1 mRNA by IL-1β in cultured RASMC. Northern blot analyses were performed with total RNA from RASMC exposed to either vehicle or IL-1β. The blots were then hybridized to HO-1 and HO-2 cDNA probes. A representative Northern blot of a time-course experiment of HO-1 stimulation by IL-1β (10 ng/ml, dose promoting maximal induction) is presented in Fig. 4B. In contrast to the more rapid in vitro induc-
tion (within 2 h), IL-1β did not increase HO-1 mRNA until 4 h after stimulation in vitro, and peak induction occurred after 24 h. The time course of HO-1 mRNA stimulation by IL-1β, including additional samples extending the stimulation to 48 h, is graphically illustrated in Fig. 4B. Twenty-four hours after the administration of IL-1β, HO-1 mRNA increased by 5.8-fold compared to vehicle. The induction of HO-1 mRNA by IL-1β decreased to 2.8-fold after 48 h. The message for HO-2, in contrast to HO-1, varied minimally after treatment with IL-1β (Fig. 4C).
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**Figure 5. Effect of IL-1β on HO-1 transcriptional rate and mRNA stability.** A. RASMC were either stimulated with IL-1β or vehicle for 24 h. Nuclei were then isolated from the RASMC, and in vitro transcription was allowed to resume in the presence of [α-32P]UTP. Equal amounts of 32P-labeled, in vitro transcribed RNA probes from each group were hybridized to 1 μg of denatured HO-1, β-actin, and HO-2 cDNA that had been immobilized on nitrocellulose filters. The bar graph represents the signal intensity of HO-1 normalized by β-actin, and the transcriptional rate was plotted as a percentage of vehicle. B. RASMC were stimulated with IL-1β (10 ng/ml) or vehicle for 12 h, then actinomycin D (5 μg/ml) was administered to the RASMC. Total RNA was extracted from the RASMC at the indicated times after administration of actinomycin D. Northern blot analyses were performed using 5 μg of total RNA/lane. After electrophoresis the RNA was transferred to nitrocellulose filters, which were hybridized to 32P-labeled HO-1 and 18 S probes. To correct for differences in loading, the signal intensity of each RNA sample hybridized to the HO-1 probe was divided by that hybridized to the 18 S probe. The normalized intensity was then plotted as a percentage of the 0-h value against time (in log scale).

**Figure 6. Effect of NOS inhibitors on the induction of HO-1 mRNA by IL-1β in RASMC.** A. RASMC were exposed to no stimulus (control), IL-1β (10 ng/ml), IL-1β (10 ng/ml) plus L-NAME (10^−5 M), L-NAME (10^−3 M) alone, IL-1β (10 ng/ml) plus SMT (10^−4 M), or SMT (10^−4 M) alone. Total RNA was extracted from the cells after 24 h of stimulation. Northern blot analyses were performed using 10 μg of total RNA/lane. After electrophoresis the RNA was transferred to nitrocellulose filters, which were hybridized to 32P-labeled HO-1 and 28S probes. B. RASMC were exposed to no stimulus (control), IL-1β (10 ng/ml), IL-1β (10 ng/ml) plus L-NAME (10^−3 M), L-NAME (10^−3 M) alone, IL-1β (10 ng/ml) plus SMT (10^−4 M), or SMT (10^−4 M) alone. Extracellular nitrite accumulation was assayed from the culture media after 24 h of stimulation. NO production was expressed as nanomoles/mg of protein. The values represent the mean ± S.D. (n = 3).

**Discussion**

CO, a gas formed endogenously from heme metabolism, shares many of the chemical and biological properties of NO (10). The physiologic importance of NO and the enzymes that foster the production of NO (NOS) have been studied in detail (5–8, 28); however, much less is known about the physiologic function of CO and the heme degradative enzymes HO-1 and HO-2. A recent study (29) showed prominent immunocytochemical staining for HO-2 in the vascular endothelium under basal conditions. HO-2 staining was also present in adventitial nerves of blood vessels and in neurons in the autonomic ganglia. The location of the staining for HO-2 was comparable with constitutive isoforms of NOS, and inhibitors of HO enzyme activity were able to partially attenuate endothelium-dependent vasodilation. This study implied complementary and possibly related roles for HO-2 and constitutive isoforms of NOS (29). Studies have also demonstrated that CO can be produced by arteries in vivo (30, 31), and higher doses of HO inhibitors (such as ZnPP, 45 μmol/kg) induce an increase in arterial
pressure in rats (32). Taken together these studies suggest CO, generated by HO-2, may contribute to the regulation of vascular tone under basal conditions. However, the role of the inducible isomerase of HO, HO-1, in vascular biology and pathophysiology is less clear.

To gain more insight into the potential role of CO in the regulation of vascular tone during endotoxic shock (a cytokine-driven disease process; Refs. 1 and 3), we analyzed the regulation of HO-1 mRNA in vascular tissue in vivo. A dramatic increase in HO-1 mRNA occurred in aortic tissue from rats receiving LPS compared with rats receiving vehicle (Fig. 1A), and this increase in message was associated with an 8.9-fold increase in HO enzyme activity (Fig. 1B). HO-2 mRNA was not increased by LPS. The increase in HO-1 protein was present in vascular smooth muscle cells and endothelial cells of both large (aorta, Fig. 2B) and small (arterioles, Fig. 2, E and F) blood vessels. The marked increase in HO enzyme activity by LPS (to a level of activity comparable with that of the liver) would suggest that HO-1-generated CO may contribute to the reduction in vascular tone during endotoxic shock.

To clarify the role of HO-1 induction in endotoxic shock, we administered ZnPP to rats that were made hypotensive by LPS. We used ZnPP instead of other metalloporphyrins to inhibit HO activity because ZnPP has been shown to be selective for HO over other microsomal enzymes (14). Moreover, ZnPP in concentrations less than 50 μmol has no inhibitory effect on NOS activity, and at a dose of 10 μmol it has no effect on soluble guanylate cyclase activity in vascular endothelial cells (29). In our experiments, ZnPP given in a dose of 10 μmol/kg abrogated the LPS-induced hypotension and it produced a significantly higher mean arterial pressure in the LPS+ZnPP group (10 μmol/kg) compared with the LPS alone group (Fig. 3). ZnPP at a dose of 1 μmol/kg prevented a further decrease in arterial pressure in rats receiving LPS, and this response was less dramatic than in the LPS-stimulated rats receiving 10 μmol/kg ZnPP. These data support the importance of HO-1, and subsequently CO, in the hemodynamic compromise of endotoxic shock.

Although our data support a detrimental role for vascular HO-1 in endotoxic shock, a recent study proposed that further induction of HO-1 by hemoglobin may protect against the oxidant damage of endotoxemia (33) by generating bilirubin (which has antioxidant properties; Ref. 34). These investigators demonstrated that the administration of HO inhibitors at doses that decrease HO enzyme activity below basal levels (SnPP and ZnPP, 50 μmol/kg) made rats more susceptible to LPS-induced death (33). Unfortunately, the available inhibitors of HO activity do not discriminate between HO-1 and HO-2. Thus, if HO-2 is an important regulator of basal vascular homeostasis (29) and an important neurotransmitter (35), we would speculate that inhibition of both HO-1 and HO-2 activity (similar to inhibition of both the constitutive and inducible pathways of NOS; Ref. 36) would be harmful during endotoxic shock. Because of this hypothesis, we chose to administer lower doses of ZnPP (10 and 1 μmol/kg) in rats after the onset of endotoxin-induced shock to prevent complete inhibition of HO enzyme activity. ZnPP given at the lower doses curtailed the LPS-induced hypotension (Fig. 3). These data suggest that the beneficial hemodynamic response to ZnPP in rats receiving LPS reflects an inhibition of inducible HO activity.

Recent preliminary reports suggest that NO itself can induce HO-1 mRNA and protein expression in cell culture (26), and we have demonstrated previously that IL-1β stimulates an increase in NO production through the inducible NOS pathway in vascular smooth muscle cells in vitro (4). Thus, to determine if the induction of HO-1 mRNA by IL-1β occurs indirectly through an increase in NO production, we stimulated vascular smooth muscle cells with IL-1β in the presence of two different NOS inhibitors. Neither L-NAME nor SMT (both of which inhibited the accumulation of NO) were able to impede the induction of HO-1 mRNA by IL-1β (Fig. 6). These data suggest the induction of HO-1 mRNA by IL-1β occurs through a NO-independent pathway in vascular smooth muscle cells.

Our study demonstrates that HO-1-derived enzyme activity can be up-regulated within vascular tissue by a pathophysiological process, endotoxemia, in vivo. In both large blood vessels (aorta) and small resistance vessels (arterioles), the increase in staining for HO-1 localized to vascular smooth muscle cells and endothelial cells. The advent of specific HO-1 antagonists, or an HO-1 gene deletion animal, would allow us to gain more insight into the pathophysiologic role of HO-1 in endotoxic shock. However, the marked induction of HO-1 enzyme activity by LPS within vascular tissue, and the beneficial hemodynamic response to ZnPP in LPS-stimulated animals, would suggest that HO-1 (and subsequently CO) contributes to the reduction in vascular tone during endotoxic shock.
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