Endogenous carbon monoxide is an endothelial-derived vasodilator factor in the mesenteric circulation

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ENDOGENOUS CARBON MONOXIDE (CO) is produced by conversion of free heme to biliverdin by the enzyme heme oxygenase (HO). HO exists as three isoforms: inducible (HO-1); constitutive (HO-2); and a third, not yet fully characterized form (HO-3). Several investigators have suggested that CO acts as an endogenously produced gaseous molecule analogous to nitric oxide (NO) (25, 26, 40). Indeed, CO has been shown to have both a vasorelaxant effect (4, 6, 12, 15, 21, 22, 41) as well as an antiproliferative effect on vascular smooth muscle (VSM) cells (20). HO-1 can be induced within the vasculature by a variety of physiological stimuli, including hypoxia (14, 20), hypertension (16, 37), endotoxic shock (42), and shear stress (39). Thus CO may be an important determinant of vascular tone under certain pathophysiological settings.

Previous work from our laboratory has shown that HO-1 protein and mRNA levels are increased in both the rat kidney and aorta after chronic hypoxia (CH) (14, 33). Functional evidence of a role for endogenous CO in regulating vascular tone has come from both whole animal (15, 33, 34) and isolated vessel preparations (4, 6, 9–12, 19, 41). For example, systemic administration of zinc protoporphyrin IX (ZnPPIX) increases renal vascular resistance in CH rats but not in control rats (33). Furthermore, exogenous administration of the HO substrate heme-L-lysinate (HLL) produces concentration-dependent increases in diameter in small mesenteric arteries from CH rats (11). In addition, our laboratory has previously shown that HO-derived CO may be important in both blunted agonist-induced vasoconstriction (4, 8–11, 33) as well as blunted myogenic reactivity (10) after CH. Interestingly, both phenylephrine (PE)-induced reactivity as well as myogenic reactivity were restored upon disruption of the endothelium (4, 9, 10). Taken together, these results suggest a potential role for CO derived from an endothelial source in the regulation of vascular tone after CH.

Although there is increasing evidence that HO-derived CO plays a role in the regulation of vascular tone, the site of HO expression within the vascular wall remains controversial. Indeed, both the constitutive and inducible isoforms of HO have been demonstrated in endothelial (29, 35, 36, 42) and VSM cells (5, 32, 42). For example, HO-1 has been shown to be expressed primarily in the medial layer of the rat aorta after lipopolysaccharide treatment (42) and within the endothelium in response to S-nitroso-N-acetyl penicillamine administration (36) as well as basally in cultured aortic VSM cells (5). Moreover, HO-2 protein has been demonstrated within the endothelium of porcine pulmonary, mesenteric, basilar, and pial arteries (35, 43) as well as in cultured aortic VSM cells (5).

Therefore, the goals of the present study were to 1) demonstrate that the HLL-induced vasodilation is due to a cellular localization of the HO responsible for these effects has not been clearly established. Therefore, we examined the response to administration of the substrate for HO, heme-L-lysinate (HLL), in endothelium-intact and endothelium-denuded small mesenteric arteries from CH male Sprague-Dawley rats. Mesenteric arteries were isolated and mounted on glass cannulas, pressurized to 60 mmHg, and superfused with physiological saline solution. All experiments were performed in the presence of 100 μM Nω-nitro-L-arginine. The vasodilator response to HLL or exogenous CO was examined. HLL experiments were performed in the presence and absence of the HO inhibitor zinc protoporphyrin IX (ZnPPIX). HLL administration resulted in a dose-dependent vasodilator response that was abolished in the presence of ZnPPIX or by endothelial removal. Exogenous CO produced a vasodilator response that was independent of an intact endothelium. Cellular localization of HO was verified through immunohistochemistry in sections of the gut and aorta from CH and control animals. Staining for HO-1, HO-2, and endothelial nitric oxide synthase was confined to the endothelium. Thus we conclude that CO is a product of HO located within the endothelium.

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to a product of the HO reaction, and 2) determine the cellular location of HO isoforms within the mesenteric circulation after CH. To this end, we hypothesized that HLL administration results in enhanced CO production through HO-1 localized within the endothelium of small mesenteric arteries from CH rats.

METHODS

Animals. All animal protocols employed in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the University of New Mexico School of Medicine. Male Sprague-Dawley rats (250–300 g body wt, Harlan Industries) were used for these experiments. CH rats were exposed to hypobaric hypoxia for 48 h (barometric pressure = 380 Torr).

Isolated mesenteric resistance artery diameter measurements. Rats were anesthetized with pentobarbital sodium (50 mg ip). A midsternal incision was made to expose the heart, and 100 units of heparin were injected directly into the left ventricle. The mesenteric arcade was excised through a midline abdominal incision and placed in ice-cold physiological saline solution (PSS; containing (in mM) 129.8 NaCl, 5.4 KCl, 0.5 NaH₂PO₄, 0.83 MgSO₄, 19 NaHCO₃, 1.8 CaCl₂, and 5.5 glucose) aerated with a gas mixture consisting of 21% O₂-6% CO₂-balance N₂. The arcade was secured in a Silastic-coated petri dish containing cold, aerated PSS. After removal of veins and adipose tissue, fourth-order arteries were transferred to a beaker of cold, aerated PSS. Arteries were then cannulated and pressurized to 60 Torr as described previously (9–11). Measurements of internal diameter (ID) were made using a high-resolution videocamera (model XC-73, Sony) and a video-tracking device (Video Dimension Analyzer V 94, Living Systems). Vessels were continuously superfused (5 ml/min) with aerated PSS warmed to 37°C and allowed to equilibrate for 60 min before any experimental manipulation. Viability of the vessel was assessed by administering PE (10⁻⁴ M). After the equilibration period, sequential doses of PE were administered to the superfusion reservoir until the vessel constricted by ~30% of baseline diameter. To reduce the possibility of interactions with NO, all experiments were performed in the presence of 100 μM Nω-nitro-l-arginine. Passive diameter was determined at the end of each experiment by superfusing the vessel in Ca²⁺-free PSS (containing (in mM) 129.8 NaCl, 5.4 KCl, 0.5 NaH₂PO₄, 0.83 MgSO₄, 19 NaHCO₃, 5.5 glucose, and 3 EGTA) for 30 min. For all experiments, changes in vessel diameter are expressed as the percent reversal of PE-induced vasoconstriction. Data were stored and subsequently analyzed on a microcomputer using a commercial data-acquisition system (CODAS, Datqa Instruments).

Specificity of HLL-induced vasodilation. Small mesenteric arteries (n = 5 arteries/group) were excised from control and CH rats, isolated, cannulated, and perfused as described above. Vessels were superfused with PSS containing 500 nM of the HO inhibitor ZnPPIX or an equal volume of drug vehicle for 1 h before experimental manipulation (n = 5 vessels/group). After the equilibration period, serial doses of HLL were administered in PE-preconstricted vessels, ranging from 10⁻⁷ to 10⁻⁵ M. Whereas ZnPPIX is a potent inhibitor of HO, at higher concentrations it may also inhibit NO synthase (NOS) and soluble guanylyl cyclase (sGC) activities (1, 13, 24). To demonstrate the lack of nonspecific effects of ZnPPIX on other heme-containing enzymes (e.g., sGC), dose-response curves to S-nitroso-N-acetyl penicillamine were performed in the presence and absence of ZnPPIX (n = 5 for the vehicle-treated group and 4 for the ZnPPIX-treated group). In addition, male Sprague-Dawley rats were treated with sodium arsenite, a known inducer of HO-1 (75 μmol/kg sc) (5), to demonstrate that the vasodilator response to HLL is due to a product of the HO reaction rather than to a factor related to hypoxia per se. Sixteen hours after sodium arsenite treatment, small mesenteric arteries (n = 5 arteries/group) were excised, isolated, and cannulated as described above. Vaso-dilator responses to HLL (10⁻⁷–10⁻⁵ M) were determined in the presence and absence of ZnPPIX in PE-preconstricted vessels.

Endothelial dependence of HLL-induced vasodilation. Small mesenteric arteries (n = 5 arteries/group) were excised from CH rats, isolated, cannulated, and superfused as described above. Vessels were superfused with PSS (without Nω-nitro-l-arginine) 1 h before experimental manipulation. After the equilibration period, sequential doses of PE were administered to the superfusion reservoir until the vessel constricted by ~30% of the initial diameter. Once a stable baseline was achieved, the response to the endothelium-dependent vasodilator ACh (10⁻⁶ M) was assessed, followed by a 15-min washout period. Arteries were denuded by passing a laser air bubble through the luminal surface (9, 10). Vascular tone was reestablished with PE (10⁻⁶ M), and the ACh response was repeated to verify endothelial removal. The vessel was then washed with PSS containing 100 μM Nω-nitro-l-arginine for 30 min. Vascular tone was reestablished with PE, and the vasodilator response to HLL (10⁻⁶ M) was assessed. In a separate set of experiments (n = 4 vessels/group), the vasodilator response to S-nitroso-N-acetyl penicillamine (10⁻⁷.5 M) was assessed in intact and denuded vessels, demonstrating that the ability of these vessels to respond to an endothelial-independent vasodilator was unaltered by passage of an air bubble through the lumen of the vessel.

Endothelial dependence on exogenous CO-mediated vasodilation. Small mesenteric arteries (n = 5 arteries/group) were excised from control and CH rats, isolated, cannulated, and superfused as described above. Disruption of the endothelium was performed in some vessels as described above. Before each experiment, 10 ml of PSS were vigorously bubbled with 100% CO gas or 100% nitrogen gas for 10 min. Vessels were preconstricted with PE to ~30% of the baseline diameter. Once a stable level of constriction was achieved, the vessel was superfused for ~2 min with a 20-fold dilution of the saturated CO solution in PSS (210 μL CO/100 ml PSS) that contained the same concentration of PE as was used to establish vascular tone. To control for pH differences between the standard PSS and the CO solution, the response to a 20-fold diluted N₂-equilibrated solution was assessed (n = 3 vessels).

Western blot analysis. Previous studies (14) have demonstrated increased HO-1 levels by Western blot in vascular tissue from CH rats compared with controls. Additional Western blots were performed to determine the level of HO-1 in the mesenteric microcirculation from control (vehicle) and sodium arsenite-treated rats (see above). Each blot consisted of the experimental group and a parallel control group. Rats were anesthetized with pentobarbital sodium (50 mg ip), and the mesenteric arcade was excised through a midline abdominal incision and placed in ice-cold dissection solution containing 30 mM MOPS, 1.2 mM NaH₂PO₄, 5 mM glucose, 2 mM piperazine, 0.02 mM EDTA (disodium salt), 145 mM NaCl, 4.7 mM KCl, 2 mM CaCl₂, 1.2 mM MgSO₄, and 10 g/l BSA; pH 7.4, 290–310 mosM). After removal of veins and adipose tissue, resistance artery branches were collected and snap frozen in liquid N₂. All vessels harvested from a single animal were pooled and treated as one sample. A microsomal
fraction was prepared using 10 mM Tris·HCl homogenization buffer (containing 255 mM sucrose, 2 mM EDTA, 12 µM leupeptin, 1 µM pepstatin A, 0.3 µM aprotinin, and 1 mM phenylmethylsulfonyl fluoride). Samples were centrifuged at 10,000 g for 10 min at 4°C. The supernatant was collected and centrifuged at 100,000 g for 60 min at 4°C. The microsomal pellet was resuspended in homogenization buffer. Sample protein concentrations were determined by the Bradford method (Bio-Rad protein assay). Proteins (20 µg/lane) were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. The blocking solution contained 5% nonfat milk, 3% BSA, 5% goat serum, 5% rabbit serum, and 0.5% Tween 20 in Tris-buffered saline (TBS) containing 10 mM Tris·HCl and 50 mM NaCl (pH 7.5). HO-1 primary antibodies were diluted in TBS plus 5% nonfat milk and 0.05% Tween 20. The secondary antibody was diluted in TBS and 0.05% Tween 20. Blots were incubated for 1 h at room temperature with goat anti-mouse horseradish peroxidase-conjugated IgG (1:5,000 dilution, Stressgen). 

Immunohistochemistry in small mesenteric arteries and aortas. Normoxic control and CH rats were anesthetized with all sections, immunoperoxidase staining of the antigen-peroxidase conjugate was achieved by treatment of sections with 3,3' diaminobenzidine tetrahydrochloride dihydrate and conjugate was prepared as described previously (33). ZnPPIX stock solution was aliquoted and stored at -80°C for a maximum of 1 wk. PE and ACh were dissolved in deionized H2O, aliquoted, and stored at -80°C until use. Sodium arsenite (100 µmol/ml) was dissolved in saline, filter sterilized using a 0.2-µm filter (Millipore), and stored at 4°C. S-nitroso-N-acetyl penicillamine was dissolved in ethanol on the day of the experiment and stored on ice until use. N≡-nitro-l-arginine was dissolved in PSS on the day of the experiment. Perfusion buffer consisted of PSS containing 2,000 units heparin, 4 mg/ml BSA, and 10^-4 M papaverine.

Results

Specificity of HLL-induced vasodilation. The magnitude of constriction generated by PE administration was 41.6 ± 10.8% (2.0 ± 0.5 µM PE) and 31.0 ± 3.5% (1.1 ± 0.5 µM PE) of the initial diameter under vehicle and ZnPPIX conditions, respectively. Changes in ID in response to HLL in pressurized mesenteric resistance arteries (60 Torr) from CH rats in the presence and absence of HO inhibition are shown in Fig. 1. HLL administration produced a dose-dependent increase in ID in resistance arteries pretreated with the vehicle for ZnPPIX. In contrast, the vasodilator response to HLL was completely abolished in the presence of HO inhibition. Because ZnPPIX may have nonspecific effects on other heme-containing enzymes (e.g., sGC) (13, 24), the vasodilator response to S-nitroso-N-acetyl penicillamine was assessed in the presence and absence of HO inhibition. As illustrated in Fig. 2, S-nitroso-N-acetyl penicillamine produced a dose-dependent increase in ID that was unaltered by ZnPPIX.

As shown in Fig. 3, HO-1 protein was detected in small mesenteric arteries from rats treated for 16 h with sodium arsenite. However, HO-1 was not detected in arteries from control animals. HLL produced a dose-dependent increase in ID in arteries from sodium arsenite-treated animals. This response was completely abolished by HO inhibition (Fig. 4).

Endothelial dependence of HLL-induced vasodilatation. The integrity of the endothelium was assessed by administration of ACh (10^-6 M) before and after passage of an air bubble through the lumen of the vessel. Figure 5 shows the response to ACh in mesenteric
There was no significant difference between groups.

Resistance arteries constricted with PE. ACh administration completely reversed the PE-induced tone. This response was inhibited in denuded mesenteric arteries, whereas the vasodilator response to S-nitroso-N-acetyl penicillamine (3 \times 10^{-7} M) was unaffected (Fig. 5).

Fig. 1. Effect of heme-l-lysinate (HLL; 10^{-7}–10^{-5} M) on internal diameter (ID) in mesenteric arteries from chronic hypoxic (CH) rats. Experiments were performed in the presence and absence of the heme oxygenase (HO) inhibitor zinc protoporphyrin IX (ZnPPIX). Fourth-order mesenteric arteries from 48-h CH rats were pretreated with N-nitro-L-arginine and either vehicle or ZnPPIX 1 h before experimentation. The change in ID is presented as the percent reversal of PE-induced constriction. Data are means ± SE (n = 5 arteries/group). *Significantly different from 10^{-7} M HLL (P ≤ 0.05); †significantly different from 10^{-5} and 10^{-7} M HLL (P ≤ 0.05); ‡significantly different from vehicle (P ≤ 0.05).

Endothelial dependence of exogenous CO-mediated vasodilation. As shown in Fig. 7, exogenous CO elicited vasodilation in small mesenteric arteries from both control and CH rats. Disruption of the endothelium did not alter this response. In contrast, superfusion with a nitrogen-equilibrated solution produced a modest transient vasoconstriction (data not shown).

Immunohistochemistry for eNOS, HO-1, and HO-2 in arteries from the rat small intestine. Photomicrographs of eNOS, HO-1, and HO-2 immunoreactivity in arteries lying within the muscularis mucosae of the small intestine from control, CH, and sodium arsenite-treated rats are illustrated in Fig. 8. An example of eNOS immunostaining in an artery from a control rat is shown in Fig. 8F. HO-1 staining in arteries from CH and control rats are shown in Fig. 8, A and B, respectively. Note that sections incubated with HO-1 primary antibody show a similar pattern of staining as that for eNOS, suggesting that HO-1 protein is localized to the endothelium. An example of HO-1 immunostaining in an artery from a sodium arsenite-treated animal is shown in Fig. 8E. Examples of HO-2-immunoreactive arteries from both control and CH rats are illustrated in Fig. 8, C and D, respectively. Again, staining for HO-2 was localized to the endothelium. No staining was observed in sections that were not treated with primary antibody (Fig. 8G).

Immunohistochemistry for eNOS, HO-1, and HO-2 in the aorta. Figure 9 contains photomicrographs of eNOS, HO-1, and HO-2 immunoreactivity in aortas from CH and normoxic control animals. Examples of eNOS immunostaining in aortas from both control and
CH rats are shown in Fig. 9, A and B. Note that sections incubated with HO-1 or HO-2 primary antibody show a similar pattern of staining as that for eNOS, suggesting that HO protein is localized to the endothelium in the aorta (Fig. 9, C–F).

**DISCUSSION**

The major findings of the present study are that 1) the vasodilator response to HLL administration is the result of a product of the HO reaction; 2) disruption of the endothelium nearly abolishes the vasodilator response to HLL; 3) exogenous CO elicited an endothelium-independent vasodilation; 4) sodium arsenite treatment increases HO-1 protein levels, 5) sodium arsenite treatment increase HO-1 protein expression in mesenteric arteries; 6) HLL causes vasodilation in vessels from sodium arsenite-treated rats, which is abolished by ZnPPIX; and 7) HO-1 and HO-2 protein is expressed within the endothelium of both small mesenteric arteries and aorta. These findings suggest that endogenous CO is an endothelium-derived vasodilator factor acting on vascular smooth muscle analogous to NO.

Several investigators have shown that HLL is a vasodilator in a number of vascular beds, including the mesenteric, cerebral, and skeletal muscle circulations (11, 16, 21, 22). For example, earlier experiments from our laboratory have demonstrated that HLL causes a dose-dependent vasodilator response in small mesenteric arteries isolated from rats exposed to 48 h of hypobaric hypoxia. However, in vessels from normoxic control animals, HLL had an effect only at the highest dose (11). The present study provides evidence that the vasodilator response to HLL is mediated by a product of the HO reaction. Indeed, HLL-induced vasodilation was abolished by ZnPPIX. This is in agreement with a study by Leffler et al. (21), who demonstrated in porcine pial arteries that HLL treatment caused a dose-dependent vasodilator response that was reversed to a vasoconstriction by the HO inhibitor chromium mesoporphyrin. Moreover, intraperitoneal injection of HLL produced a decrease in blood pressure in spontaneously hypertensive rats that was prevented by pretreatment with the HO inhibitor zinc deuteroporphyrin. Furthermore, hemin-induced increases in HO activity in renal microsomes was abolished by the metalloporphyrin tin mesoporphyrin (7).

Taken together, the results of these studies suggest that hemin-induced vasodilation is mediated by a product of the HO reaction, presumably CO.

It is well established that catabolism of free heme by HO results in the production of equimolar quantities of CO, free iron, and biliverdin. Previous work from our laboratory has provided evidence that the vasoactive product of the HO reaction is CO. Indeed, neither free iron nor biliverdin reduced the active tension developed in PE-preconstricted aortic rings, whereas exogenous CO produced a dose-dependent reduction in tension (4). Moreover, others have shown that treatment
of rat aortic VSM cells with hemin resulted in a dose-dependent increase in CO release (31). Taken together, these results suggest that the administration of HLL augments the production of CO by the enzyme HO.

The present study provides evidence that HO-1 and HO-2 are present within the endothelium of mesenteric arteries from both normoxic and hypoxic animals. Previously, HO-1 protein has been shown within the endothelial layer of small pulmonary arteries in response to hemin treatment (2) as well as in the aorta after high concentrations of S-nitroso-N-acetyl penicillamine (36). However, others have shown HO-1 within the medial layer of the aorta (42) and small pulmonary arteries (3) in response to lipopolysaccharide and hypoxia, respectively. In addition, HO-1 mRNA has been demonstrated in cultured aortic and pulmonary VSM cells in response to hypoxia (32). Furthermore, HO-1 expression has also been shown in aortic VSM cells after sodium arsenite treatment (5). These inconsistencies may be explained by differences between vascular beds, the factors used to induce HO-1, or cell culture conditions. The present study provides evidence that within the mesenteric circulation both HO-1 and HO-2 are expressed selectively within the endothelium in tissue from both control and CH animals. In contrast, others have failed to demonstrate HO-1 expression under control conditions (3, 36, 42) using immunohistochemistry. These differences as well as the inability of the present study to detect differences between control and hypoxic tissue may be due to low levels of HO-1 in these vascular beds or to a lack of adequate sensitivity of the immunohistochemistry technique.

We have previously shown in aortic rings from CH rats that the contractile response to PE after inhibition on NOS and HO was greater than in tissue treated only with Nω-nitro-L-arginine (4). Removal of the endothelium abolished the effect of HO inhibition in this tissue, providing support for the postulate that HO-derived CO is an endothelial-derived vasoactive compound.

Fig. 8. Immunohistochemistry for HO-1, HO-2, and endothelial NO synthase (eNOS) in mesenteric arteries from CH and control rats. A and B: examples of HO-1 staining in mesenteric arteries from CH and control rats, respectively. C and D: mesenteric arteries immunostained for HO-2 from control and CH rats, respectively. E: example of HO-1 staining in a mesenteric artery from a sodium arsenite-treated rat. F: immunostaining for eNOS in a mesenteric artery from a control rat. G: mesenteric artery from a sodium arsenite-treated rat incubated with horse anti-mouse IgG secondary antibody only. Note that HO-1 and HO-2 have a similar pattern of staining as that of eNOS. Magnification: ×40; bar = 20 μm.

Fig. 9. Immunohistochemistry for HO-1, HO-2, and eNOS in aortas from CH and control rats. A and B: examples of HO-1 staining in aortas from CH and control rats, respectively. C and D: aortas immunostained for HO-2 from CH and control rats, respectively. E and F: immunostaining for eNOS aortas from a CH and control rats, respectively. Note that HO-1 and HO-2 have a similar pattern of staining as that of eNOS. Magnification: ×20.
CO has been shown to inhibit the synthesis of endothelial-derived vasoactive compounds (18). Hence, the vasodilator response to endothelium-derived CO may be mediated through an autocrine effect on endothelial cells rather than acting directly on VSM cells. In the present study, administration of exogenous CO produced a vasodilator response in small mesenteric arteries. Removal of the endothelium did not alter this response, suggesting the CO-mediated vasodilation occurs through a direct effect on VSM cells. In addition, the vasodilator response to CO in arteries from control and CH rats was similar. Therefore, exposure to an hypoxic environment does not alter the sensitivity of these vessels to CO.

Given that ZnPPIX inhibits both HO-1 and HO-2 and that both isoforms are expressed in small mesenteric vessels, it is not possible to know with certainty which isoform is being affected in our preparation. Indeed, HO-2 has been shown to be abundant in the brain, testes, and endothelial lining of the aorta (43). However, this isoform is considered to be constitutive, although its expression can be induced by glucocorticoids (27). Furthermore, we have failed to observe any effect of hypoxic exposure on HO-2 mRNA or protein in arterial and aortic tissue in earlier studies from our laboratory (34). Given that hypoxia induces HO-1 expression (3, 20, 32), and the inability of HLL to cause vasodilation in vessels from control animals except at high concentrations (11), elevated levels of this form of the enzyme are most likely responsible for the observed response to HLL in our experiments. Indeed, we have previously shown that 48 h of hypoxia induces HO-1 expression in the rat aorta (14). Moreover, in the present study, sodium arsenite treatment resulted in an increase in HO-1 protein expression within the mesenteric vasculature. Administration of HLL caused vasodilation in these vessels, but not in vessels from control animals (11), even though both HO-1 and HO-2 are expressed within the mesenteric vasculature. These results suggest that HO-1 is the isoform responsible the vasodilator response to HLL.

As stated above, we have previously shown a differential response to HLL between control and CH rats. The vasodilator response to excess substrate (i.e., HLL) implies a substrate limitation in vessels from CH rats. This may result from an increase in expression of HO-1 or an increase in enzyme activity after hypoxic exposure. Alternatively, de novo synthesis of heme within endothelial cells may be impaired in CH rats, resulting in a reduced availability of free heme.

Finally, Johnson and Johnson (17) have suggested that CO acts as a vasoconstrictor agent in endothelium-intact vessels through the inhibition of NOS, whereby the vasodilator properties of CO are unmasked when the endothelium is removed or NOS is inhibited. However, because our experiments eliminated the contribution of NO by administration of a NOS inhibitor, any potential interactions between CO and NO have been eliminated. Furthermore, we have previously shown that exogenous CO relaxed both endothelium-intact and denuded aortic rings (4). Discrepancies between the results of our laboratory and those of Johnson and Johnson (17) may be explained by differences in vascular beds studied or to other changes independent of HO occurring at the level of the vasculature secondary to hypoxic exposure. In addition, NOS inhibition also rules out any nonspecific actions of ZnPPIX on NOS activity in the present studies.

In summary, the current study provides evidence that HLL administered to small mesenteric arteries results in vasodilation mediated by CO produced by HO located within the endothelium. Further studies are required to better elucidate the downstream target(s) involved in CO-mediated vasodilation.

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