Renal vasodilatory influence of endogenous carbon monoxide in chronically hypoxic rats

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O’Donaughy, Theresa L., and Benjimen R. Walker. Renal vasodilatory influence of endogenous carbon monoxide in chronically hypoxic rats. Am J Physiol Heart Circ Physiol 279: H2908–H2915, 2000.—Chronic hypoxia (CH) attenuates systemic vasoconstriction to a variety of agonists in conscious rats. Recent evidence suggests that similarly diminished responses to vasoconstrictors in aortic rings from CH rats may be due to increased endothelial heme oxygenase (HO) activity and enhanced production of the vasodilator carbon monoxide (CO). Thus we hypothesized that a hypoxia-induced increase in HO activity is responsible for decreased vasoconstrictor responsiveness observed in conscious CH rats. CH (4 wk at 0.5 atm) and control rats were renal denervated and instrumented for the measurement of renal blood flow (RBF) and blood pressure. First, renal vasoconstrictor responses to graded intravenous infusion of phenylephrine (PE) were assessed in conscious rats. CH rats demonstrated significantly diminished renal vasoconstrictor responses to PE compared with control responses that persisted even with acute restoration of normoxia. In additional experiments, CH rats exhibited increased renal vascular resistance and decreased RBF in response to the HO inhibitor zinc protoporphyrin IX (11 μmol/kg iv), whereas renal hemodynamics were unaffected by the inhibitor in control animals. Furthermore, we demonstrated greater HO enzyme activity in renal tissue from CH rats compared with controls. These data suggest that enhanced HO activity contributes a tonic vasodilatory influence in the renal vasculature of CH rats that may be responsible for the diminished sensitivity to vasoconstrictor agonists observed under these conditions.

Effects of sustained hypoxia are not immediately reversible and are thus unrelated to the well-established acute relaxant actions of this stimulus. The mechanism(s) responsible for diminished responsiveness after CH have not been clearly defined but may be related to the enhanced tonic release of a local vasodilator. In support of this possibility, earlier work suggests that inhibition of endothelial heme oxygenase (HO) production of the vasodilator carbon monoxide (CO) restores reactivity in aortas from CH rats.

H0s are microsomal enzymes responsible for degrading heme to biliverdin, releasing free iron and CO (39, 40). There are three known isoforms of these ubiquitously expressed enzymes (24, 25). The HO-1 isoform is induced by a wide variety of stimuli, including hypoxia, hyperoxia, and oxidative stress, and is also known as heat shock protein 32 (18, 37). Its promoter contains several regulatory sites including a hypoxia-inducible factor-1 (HIF-1) binding site (21). HO-1 is expressed in vivo in the vascular wall and in cultured endothelial cells and vascular smooth muscle cells (VSMC) (6, 26, 27, 35). Furthermore, HO-1 expression is enhanced under hypoxia conditions in VSMC (26). CO produced by activity of this enzyme is thought to elicit responses analogous to that of NO, readily crossing the sarcolemmal membrane to activate VSMC soluble guanylyl cyclase (sGC), thereby causing relaxation (3, 35).

The current study was designed to test the hypothesis that enhanced in vivo HO activity contributes a tonic vasodilatory influence in the renal bed of CH rats. Our data show that HO enzyme activity is increased in renal tissue from CH rats, which correlates with reduced vasoconstrictor responses to phenylephrine. Furthermore, we demonstrate a dramatic renal vasoconstrictor response to inhibition of HO in CH rats but not in controls. These results suggest that CO exerts a tonic renal vasodilatory effect in this setting that may account for reduced responsiveness to vasoconstrictors.

METHODS

All procedures 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.

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Experimental groups. Chronically instrumented, unrestrained rats were used for all conscious animal experiments. Experiments were performed on three groups of rats: one group of CH rats; one group of CH rats returned to a normoxic environment; and one group of normoxic rats. Chronically hypoxic rats were housed for 4 wk in a hypobaric chamber in which the barometric pressure was maintained at 240 ± 10 mmHg below ambient pressure. The chamber was opened three times per week to provide animals with fresh food, water, and bedding.

Surgical preparation. Male Sprague-Dawley rats (265–400 g) were chronically instrumented with renal pulsed-Doppler flow probes. The flow probes were constructed by forming Silastic cuffs around piezoelectric crystal assemblies (20 MHz, 1-mm diameter; Crystal Biotech). Rats were anesthetized with a mixture of ketamine (91 mg/kg im) and acepromazine (0.9 mg/kg im). A midline laparotomy was performed, and the left renal artery was isolated. The left kidney was denervated by severing visible renal nerves and by swabbing the renal artery with a solution of 20% phenol in ethanol. This method has been shown to produce effective renal denervation as assessed by significant reduction of tissue catecholamine content (17, 33) and was performed to eliminate the influence of reflex alterations in vascular tone at the time of study. The Doppler flow probe was placed around the artery and secured in place without affecting flow. Flow probe leads were passed through the abdominal wall and routed subcutaneously to the base of the neck, where they were exteriorized and placed in a protective plastic cap sutured to the skin. The abdominal cavity was closed, and the animals were treated with systemic and topical antibiotics. CH rats underwent this surgery after 3 wk of hypobaric hypoxia and were returned to the hypoxic chamber within 24 h of the procedure. After a recovery period of 5 days, the animals were reanesthetized as described above. Polyethylene catheters (2× PE-10 and PE-50) were advanced into the abdominal vena cava and aorta via the femoral vein and artery, respectively. The catheters were routed subcutaneously and placed in the protective cap with the flow probe leads. Systemic and topical antibiotics were again administered. CH rats were returned to the hypobaric chamber when recovered from anesthesia. Animals were given 2–3 days to recover from this procedure before experiments were performed.

Conscious animal experiments. On the day of each experiment, rats were placed in a Plexiglas container (23 × 14 × 10 cm) large enough to allow free movement but small enough to discourage excessive exploration. Fresh bedding covered the bottom of the chamber. The catheters and Doppler probe leads were fed out of the top of the box, and the catheters were opened and flushed with heparinized saline. The arterial catheter was connected to a Statham-Gould P22 Gb pressure transducer with the output amplified by a Gould Universal amplifier. Doppler leads were connected to a Crystal Biotech Doppler flowmeter for measurement of renal blood flow (RBF). Pulsatile and mean arterial blood pressure (MAP, in mmHg), heart rate, and pulsatile and mean RBF Doppler signals (kHz of Doppler shift) were continuously recorded on separate channels of a Gould RS 3800 chart recorder. The Doppler methodology employed in this study does not permit a measure of actual flow but, rather, presents a measure of relative change in renal blood flow. All signals were simultaneously processed with an analog-to-digital converter and stored on a computer for later analysis. Animals were allowed 30–60 min to adjust to their environment, and experiments were initiated when rats demonstrated stable blood pressure and heart rate. During equilibration, room air or 12% O2, mimicking the PO2 in the hypobaric chamber, was continuously circulated through the Plexiglas container.

Renal vasoconstrictor responses to phenylephrine. Previous experiments have documented that CH exposure results in diminished systemic vasoconstrictor reactivity in conscious rats that persists on acute return to a normoxic environment (5). However, the specific vascular beds contributing to this response have not been determined. To assess whether the renal vascular bed demonstrates attenuated vasoconstritor reactivity on exposure to CH, we performed experiments to determine the renal vasoconstrictor response to increasing doses of phenylephrine (PE) in control and CH rats. Measurements were made under normoxic conditions for both the control (n = 5) and CH (n = 7) groups and also under hypoxic conditions (n = 6) for the CH group. Where gas conditions were not changed, 10 min of baseline data were collected after the equilibration period, followed by a graded infusion of PE at 3, 6, and 9 μg·kg⁻¹·min⁻¹ iv for 3 min at each rate. Steady-state renal hemodynamic responses were achieved at each rate. For experiments involving the acute return of CH rats to normoxia, an additional 20 min were allowed to reestablish equilibrium, and 5 min of baseline data were collected under normoxic conditions before the start of the PE infusion.

Effect of HO inhibition on renal hemodynamics. These experiments were designed to assess whether inhibition of HO differentially affects renal vascular resistance in control versus CH rats. CH (n = 7) and control rats (n = 6) were surgically instrumented as described in Surgical preparation. Renal hemodynamic measurements were made in the presence of the HO inhibitor zinc protoporphyrin IX (ZnPPIX) or its vehicle. Vehicle controls were performed on the first day of experimentation, and the ZnPPIX treatment was performed on the second day, because ZnPPIX administration may subsequently alter HO mRNA and protein levels (34). After animals equilibrated to their environment, 10 min of baseline data were collected. ZnPPIX (11 μmol/kg) or its vehicle was infused intravenously over a 5-min period. After administration, hemodynamic measurements were monitored for an additional 20 min.

HO enzyme assay. HO activity was assessed by using modifications of previously published methods (28, 38). The assays were conducted as paired experiments. Briefly, kidneys were harvested from one control and one CH rat (n = 6 for each group), along with the liver from the control animal. Tissue was homogenized, and the microsomal fraction was obtained. The supernatant from the control liver sample was used as a source of biliverdin reductase for the assay. The total amount of protein added was determined at the end of the experiment. The kidney microsomal pellets were resuspended in 100 mM potassium phosphate buffer (pH 7.4) containing 2 mM MgCl2. A reaction mixture containing 100 mM potassium phosphate, 2 mM MgCl2, 0.8 mM NADPH, 2.0 mM glucose-6-phosphate (G-6-P), 0.0016 U/μl glucose-6-phosphate dehydrogenase (G-6-PD), 400 μM hemin, and ~2 mg of control liver supernatant protein was preincubated in the dark on ice for 30 min. Two to six milligrams of microsomal protein were added to the reaction, bringing the final reaction volume to five hundred microliters. The reaction was incubated for 0 or 60 min at 37°C in the dark and stopped by the addition of 500 μl of chloroform. Samples were vortexed, microcentrifuged at 10,000 g at room temperature for 10 min, and then maintained at room temperature in the dark for 2 h. A portion of the organic phase was removed to measure bilirubin formed by calculating the difference in absorption between 464- and 530-nm wavelengths by using an extinction coefficient of 40 mM⁻¹·cm⁻¹. HO activity was expressed
as picomoles of bilirubin formed per milligram of protein per hour. Protein assays were performed according to the Lowry method by using a Bio-Rad kit on both the pellet and the supernatant.

Preparation of experimental solutions. PE (Sigma) was dissolved in 0.9% saline, aliquoted, and frozen until use. ZnPPIX (Porphyrin Products) solutions were prepared as an adaptation of methods described by Vreman et al. (43). Specifically, 50 mg of ZnPPIX were solubilized in 500 μl of 10% ethanolamine. Two milliliters of 0.9% NaCl were slowly added, and then 1 M HCl was used to bring the pH to 7.6–8.0. The solution was brought to final volume of 5 ml with H2O and was filter sterilized with a 0.2-μm filter. Because ZnPPIX is a photosensitive compound, solutions were prepared, and experiments performed, in reduced light. Spectrophotometric measurements were performed to verify final ZnPPIX concentration. The solution was prepared the day of experiment and stored at 4°C until use. For the HO enzyme assay, 400 μM hemin was dissolved in 50% DMSO, 8 mM NADPH in 10 mM NaOH, and 20 mM G-6-P and 0.016 U/μl G-6-PD in distilled, deionized H2O. All reagents were purchased from Sigma, and all solutions except G-6-P were prepared on the day of the experiment.

Calculation and statistics. Renal vascular resistance (RVR) was calculated at 5-min intervals by dividing MAP by RBF. Venous pressure is assumed to be zero for the purposes of estimating the pressure gradient across the renal bed. Because Doppler flow probes were not calibrat ed for actual blood volume flow, all RBF and RVR data are expressed as percentages of initial values. Data were analyzed using paired and unpaired Student’s t-tests and one- and two-way ANOVA followed by Student-Newman-Keuls post hoc tests where applicable. Data are reported as means ± SE. P < 0.05 was considered statistically significant.

RESULTS

Renal vasoconstrictor responses to PE. Table 1 presents baseline MAP and heart rate for the different groups of rats. CH rats maintained a higher MAP than control rats, even on return to normoxia, as demonstrated in earlier work (5). Figure 1 shows the MAP, heart rate, RBF, and RVR responses to graded infusions of PE in control (n = 5), CH (n = 6), and CH rats returned to normoxia for 20 min before the start of PE infusion (n = 7). There were no significant differences in the heart rate response to PE among the three groups of rats (Fig. 1B); however, the pressor responses of both groups of CH rats were significantly less than that of control rats (Fig. 1A). Furthermore, there was a slight but significant difference between the pressor responses of the two CH groups at the two highest doses of PE. The RBF response to PE in CH rats breathing 12% O2 was greatly diminished compared with that in control rats (Fig. 1C). Correspondingly, the RVR response of CH rats was significantly blunted compared with that of control rats (Fig. 1D). Furthermore, attenuated vasoconstrictor responsiveness persisted in CH rats on acute return (20 min) to a normoxic (21% O2) environment before infusion. These data establish that the renal bed appears to be a major contributor to the attenuated systemic vasoconstrictor reactivity previously observed in CH rats.

Effect of HO inhibition on renal hemodynamics. Figure 2 shows MAP, heart rate, RBF, and RVR responses to intravenous administration of the HO inhibitor ZnPPIX in control (n = 6) and CH (n = 7) rats. Baseline MAP for control and CH rats was not different between groups (109 ± 4 and 121 ± 7 mmHg, respectively); however, baseline heart rate was significantly higher in CH compared with control rats (374 ± 15 vs. 378 ± 15 beats/min, respectively). There were no significant differences in the change in MAP or heart rate between these groups over the course of the experiment, although there was a transient increase in MAP (P = 0.051) 10 min after the start of ZnPPIX infusion in controls, as previously described by others (16). A significant reduction in RBF in CH but not control rats was observed 15–20 min after infusion (Fig. 2C). Consistent with the blood flow response, there was a corresponding increase in calculated RVR in CH but not control rats in response to ZnPPIX administration (Fig. 2D). The maximal reduction in RBF in CH rats was reached ~20 min after the start of the ZnPPIX infusion. Baseline MAP (107 ± 6 and 129 ± 6 mmHg) and heart rate (374 ± 7 and 436 ± 18 beats/min) were greater in CH than in control rats, as observed in a previous study (5). Table 2 presents data from parallel control experiments indicating that the vehicle for ZnPPIX had no effect on renal hemodynamics in control or CH rats.

HO activity in kidneys from CH and control rats. Figure 3 compares relative HO activity in renal tissue from control and CH rats (n = 6 in each group). Data from paired experiments demonstrate consistently greater bilirubin formation in the microsomal fraction of kidney tissue from CH compared with control rats.

DISCUSSION

The major findings of this study are that 1) the denervated renal vascular bed of CH rats demonstrates attenuated vasoconstrictor reactivity in response to PE compared with that of control rats, which is maintained on acute return to a normoxic environment; 2) administration of the HO inhibitor ZnPPIX causes decreased RBF and increased RVR in CH but not control rats; and 3) HO enzyme activity is increased in renal tissue from CH compared with control rats. These data suggest that HO activity is increased in CH rats and that CO produced by this reaction exerts a tonic vasodilatory influence in the renal bed under these conditions.

Several groups have shown that animals exposed to CH demonstrate diminished responsiveness of the sys-
temic vasculature to a variety of vasoconstrictors (5, 8, 14, 15). Furthermore, this attenuated vasoconstriction is maintained even on acute return to a normoxic environment, as previously demonstrated in both conscious animal and isolated vascular ring studies (4, 5). Further experiments are needed to establish the duration of attenuated vasoconstriction on return to normoxia. Our study illustrates a dramatic reduction in the RVR response to the vasoconstrictor PE in CH rats either maintained in hypoxia or acutely restored to normoxia for 20 min. These findings suggest that the renal bed is a significant contributor to the diminished systemic vasoconstrictor response under these conditions. The presence of reduced responsiveness in the renal bed after sympathetic denervation indicates that CH-induced attenuation of systemic vasoconstrictor reactivity is influenced by factors present at the level of the vasculature and is not a reflex response. This

Fig. 1. Systemic and renal hemodynamic responses to increasing doses of phenylephrine (PE; 3, 6, and 9 μg·kg⁻¹·min⁻¹ iv) in control rats (●, n = 5) and in chronic hypoxia (CH) rats breathing either 12% O₂ (○, hypoxic, n = 6) or 21% O₂ (●, normoxic, n = 7). A: mean arterial pressure (MAP); B: heart rate; C: renal blood flow (RBF); D: renal vascular resistance (RVR). Data are expressed as means ± SE. *Significant difference from control; #significant difference from normoxic CH rats.

Fig. 2. Systemic and renal hemodynamic responses to a 5-min intravenous infusion of ZnPPIX (11 μmol/kg) in control (○, n = 6) and CH (●, n = 7) rats. A: MAP; B: heart rate; C: RBF; D: RVR. Data are expressed as means ± SE. *Significant difference from control.
CH rats become slightly hypocapnic due to enhanced CO2 drive associated with diminished bicarbonate levels. Although arterial blood gases differ somewhat between CH and control rats breathing normoxic gas mixtures, it is unlikely that these differences are responsible for the altered reactivity to PE observed in our studies. Indeed, previous work (4) demonstrated that isolated vascular rings from CH rats display attenuated contractility compared with controls when studied under identical pH, PO2, and PCO2 conditions.

Several hypotheses have been advanced concerning the mechanism of attenuated systemic vasoconstrictor responsiveness after CH. One theory is that CH is associated with altered VSMC signaling in response to pressor agents. For example, decreased α1-adrenergic receptor density and affinity may occur after CH and may be partly responsible for diminished vasoreactivity (10, 46). In addition, CH is associated with attenuated coupling efficiency of α1-adrenoceptors to inositol (1,4,5)-trisphosphate (IP3) and tissue contractile sensitivity to IP3 (11, 12, 42). CH exposure has also been shown to inhibit Ca2+ mobilization and myofilament sensitivity in response to 5-hydroxytryptamine in uterine arteries (47). Although these studies suggest altered VSMC signaling responses to vasoconstrictor agonists after CH, an alternative hypothesis for diminished reactivity could be the enhanced local release of a vasodilator under these conditions.

In support of this latter hypothesis, recent data suggest that augmented release of an endothelium-derived vasodilator may contribute to diminished vascular contractile reactivity (4, 8). Harrison and co-workers (8) provided evidence for the involvement of a cyclooxygenase product in reduced vasoconstrictor responsiveness in CH guinea pigs. In addition, increased expression of endothelial nitric oxide synthase (eNOS) occurs in the rat pulmonary circulation during CH (13, 20, 32), although it is unclear whether a similar effect occurs in the systemic vasculature. Indeed, blockade of NOS activity in isolated aortic rings from CH rats did not normalize contractile responses to similarly treated control groups (4). In contrast, earlier work from our laboratory demonstrated that, in NOS-inhibited aortic rings, further inhibition of HO resulted in equal contractile responses in vessels from CH and control rats, suggesting that CO may act as a tonic vasodilator in this setting.

Table 2. Renal hemodynamics for ZnPPIX vehicle-treated control and CH rats

<table>
<thead>
<tr>
<th>Time Postinfusion, min</th>
<th>ΔMAP, mmHg Control CH</th>
<th>ΔHeart Rate, beats/min Control CH</th>
<th>RBF, %Control Control CH</th>
<th>RVR, %Control Control CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6.4±3.6</td>
<td>3.3±1.8</td>
<td>8.0±13.1</td>
<td>3.7±6.6</td>
</tr>
<tr>
<td>10</td>
<td>2.8±2.0</td>
<td>−0.3±1.3</td>
<td>1.6±8.3</td>
<td>1.5±8.6</td>
</tr>
<tr>
<td>15</td>
<td>3.4±1.0</td>
<td>0.2±1.7</td>
<td>6.0±6.9</td>
<td>2.5±8.6</td>
</tr>
<tr>
<td>20</td>
<td>−1.2±0.7</td>
<td>−2.7±1.3</td>
<td>−2.8±4.5</td>
<td>−7.3±6.1</td>
</tr>
<tr>
<td>25</td>
<td>0.2±2.0</td>
<td>−1.7±1.8</td>
<td>−7.4±2.4</td>
<td>−3.8±10.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Δ, change; RBF, renal blood flow; RVR, renal vascular resistance; ZnPPIX, zinc protoporphyrin IX.

Conclusion is further supported by several studies demonstrating diminished contractility in isolated vascular preparations from CH rats compared with those from control rats (4, 5, 8, 15).

Vasoconstrictor responses to infused agonists such as PE are comprised of direct agonist effects on vascular smooth muscle and secondary myogenic constriction due to the associated pressor response. Because acute hypoxia has been suggested to impair renal autoregulation (41), it is possible that attenuated reactivity to PE could be due to removal of this secondary component of vasoconstriction. However, previous studies (7) performed on conscious rats with controlled renal perfusion pressure concluded that the relative contribution of the myogenic component to PE-induced renal constriction is unchanged by acute hypoxic exposure. We interpret the fact that CH rats exhibit both a reduced pressor response and a reduced fall in RBF in response to PE as evidence that the renal bed participates in blunted agonist-induced vasoconstriction in this setting. Nevertheless, it is still possible that prolonged hypoxic exposure could affect the myogenic responsiveness to this vascular bed, thereby influencing our results.

Although arterial blood gases were not determined in the present study, previous work (5) showed that rats exposed to 4-wk CH exhibit the predicted compensated respiratory alkalosis. On returning to normoxia, arterial blood gases differ somewhat between CH and control rats breathing normoxic gas mixtures, it is unlikely that these differences are responsible for the altered reactivity to PE observed in our studies. Indeed, previous work (4) demonstrated that isolated vascular rings from CH rats display attenuated contractility compared with controls when studied under identical pH, PO2, and PCO2 conditions.

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HO activity has been linked to vasodilation under normal and pathophysiological conditions. Johnson et al. (16) observed a pressor response to HO inhibition in control rats; however, this effect appeared to be due to altered sympathetic nerve activity rather than a local vascular effect. To eliminate confounding influences of sympathomodulation, we performed experiments on renal denervated animals and failed to detect a renal vasoconstrictor effect of ZnPPIX in control animals. However, we did observe a transient increase in blood pressure and heart rate after administration of the inhibitor in these rats that was similar to that observed by these earlier investigators. In a more recent study, Kozma et al. (19) provided evidence that locally produced CO may act as an inhibitory modulator of myogenic tone in pressurized skeletal muscle arteries; however, our experiments did not produce evidence for this phenomenon in the denervated renal vasculature of control animals.

Our data strongly suggest that CH enhances HO activity that, in turn, promotes vasodilation. The most likely source of this effect is through production of CO. Indeed, in a previous study, Caudill et al. (4) found that the other products of heme oxygenase activity, i.e., iron and biliverdin, did not attenuate contractile reactivity in aortic rings, whereas CO elicited dose-dependent relaxation. There is evidence that CO causes vasorelaxation through multiple pathways. Caudill et al. (4) found that the relaxant effect of CO in aortic rings was totally blocked by sGC inhibition; however, other investigators have described non-cGMP-dependent effects of CO. For example, Wang and Wu (44) observed that exogenously applied CO reduced current through Ca²⁺-activated potassium channels (KCa) putatively by chemically modifying the extracellular domain of these channels. Other investigators have confirmed involvement of KCa channels in the vasodilatory effects of endogenous CO (22). Thus CO-induced vasodilation may involve multiple signaling pathways.

In contrast to the results from control rats, CH animals demonstrated significant renal vasoconstriction after administration of HO inhibitor, suggesting that inhibition of HO differentially affects renal hemodynamics in CH versus control rats. These results are consistent with other reports in the literature documenting enhanced vasodilatory influence from HO activity in pathophysiological situations associated with increased expression of the enzyme. For example, Yet et al. (45) showed that administration of lipopolysaccharide to rats caused increased HO-1 expression in vascular tissue as well as elevated enzyme activity. Administration of ZnPPIX prevented the development of hypotension in these animals, suggesting that endogenous CO plays a major role in endotoxins shock. In other experiments, Seki et al. (36) recently demonstrated that ZnPPIX caused a marked increase in blood pressure in stroke-prone spontaneously hypertensive rats (SHR). The inhibitor affected blood pressure to a much lesser degree in control rats, suggesting that HO expression is elevated in SHR. This possibility was supported by observation of increased HO-1 and HO-2 mRNA in the aorta of SHR compared with that in controls. Furthermore, the selective pressor effect of ZnPPIX was maintained in the presence of ganglionic blockade in SHR, further suggesting a role of locally produced CO in the control of blood pressure in these animals. Our observation of a selective effect of ZnPPIX only in CH rats suggests that this HO inhibitor is not likely affecting other heme-containing enzymes, such as sGC and NOS, and is consistent with increased expression of HO after hypoxia. ZnPPIX has been shown to be a specific inhibitor of HO at the concentrations utilized in this study (1, 23). Furthermore, the fall in RBF observed after administration of ZnPPIX in CH rats is not likely due to different basal flow in these animals because RBF has been shown to be unaffected by CH in the rat (29). These data suggest that, in a normoxic environment, endogenous CO does not play a significant role in regulation of renal vascular tone. However, after CH, there may be an increased reliance on this vasodilator as observed in other pathophysiological conditions.

The selective effect of HO inhibition on renal hemodynamics between CH and control rats is consistent with our observation of greater renal HO enzyme activity in tissue from CH animals. Ou and Smith (30) reported a similar elevation in renal as well as hepatic HO enzyme activity in CH rats compared with controls. Although this earlier report documented apparent hypoxia-induced hemoglobinemia, the enzyme activity measurements in the present study were conducted with excess substrate present in each reaction. Thus variability in possible in vivo substrate availability does not account for the observed differences in activity between groups under these controlled conditions. Because the protocol included the use of the cytosolic fraction from the liver of the control rat in the pair as a source of necessary cofactors for this reaction, experiments were performed in a pairwise fashion. Therefore, although variability was observed in the levels of enzyme activity in renal tissue from control rats, HO activity was consistently 1.5–2.0 times higher in CH compared with control tissue under identical conditions. These data strongly suggest that the amount of HO enzyme in renal tissue is greater in CH compared with control rats; however, the current experiments do not delineate the cell type(s) responsible for this observation or the mechanism for this apparent upregulation.

HO-1 is a highly inducible gene, and several factors associated with CH could potentially influence its transcription. For example, numerous studies have shown increased HO-1 transcript after hypoxic exposure in different tissues and in cultured cells (6, 21, 26, 27, 35). Hypoxic induction of HO-1 has been linked to the transcription factors HIF-1 and AP-1 and may depend on cell type (9, 21). An alternative HO-1 stimulus could be hypoxia-induced hemoglobinemia (30). HO-1 is induced by the enzyme substrate heme (38); therefore, increased free hemoglobin reported during CH (30) could be responsible for increased HO-1 transcription. Other studies suggest that NO may stimulate in-
increased HO activity in endothelial cells (27) and VSMC (6). Because eNOS expression is elevated in some vascular beds in response to CH (31), it is possible that enhanced vascular NO production could be involved in HO-1 induction under these conditions. A final possibility is that the CH-induced increase in HO activity is not regulated by increased gene transcription but, rather, by enhanced enzyme stability or rate of translation. Further studies are needed to address these possibilities.

In summary, we have shown that the renal vascular bed is a major contributor to the previously described CH-induced reduction in systemic vasoconstrictor activity. Furthermore, endogenous CO appears to exert a tonic renal vasodilatory influence in this setting.

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