Cardioprotective and vasomotor effects of HO activity during acute and chronic hypoxia

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Hartsfield, Cynthia L., Ivan F. McMurtry, D. Dunbar Ivy, Kenneth G. Morris, Shanda Vidmar, David M. Rodman, and Karen A. Fagan. Cardioprotective and vasomotor effects of HO activity during acute and chronic hypoxia. Am J Physiol Heart Circ Physiol 287: H2009–H2015, 2004.—Prolonged hypoxia leads to the development of pulmonary hypertension. Recent reports have suggested enhancement of heme oxygenase (HO), the major source of intracellular carbon monoxide (CO), prevents hypoxia-induced pulmonary hypertension and vascular remodeling in rats. Therefore, we hypothesized that inhibition of HO activity by tin protoporphyrin (SnPP) would exacerbate the development of pulmonary hypertension. Rats were injected weekly with either saline or SnPP (50 μmol/kg) and exposed to hypobaric hypoxia or room air for 5 wk. Pulmonary and carotid arteries were catheterized, and animals were allowed to recover for 48 h. Pulmonary and systemic pressures, along with cardiac output, were recorded during room air and acute 10% O2 breathing in conscious rats. No difference was detected in pulmonary artery pressure between saline- and SnPP-treated animals in either normoxic or hypoxic groups. However, blockade of HO activity altered both systemic and pulmonary vasoreactivity to acute hypoxic challenge. Despite no change in baseline pulmonary artery pressure, all rats treated with SnPP had decreased ratio of right ventricular (RV) weight to left ventricular (LV) plus septal (S) weight (RV/LV + S) compared with saline-treated animals. Echocardiograms suggested dilatation of the RV and decreased RV function in hypoxic SnPP-treated rats. Together these data suggest that inhibition of HO activity and CO production does not exacerbate pulmonary hypertension, but rather that HO and CO may be involved in mediating pulmonary and systemic vasoreactivity to acute hypoxia and hypoxia-induced RV function.

Heme oxygenase; carbon monoxide; pulmonary hypertension; right ventricular hypertrophy

Heme oxygenase (HO) is the rate-limiting enzyme in the degradation of heme to biliverdin releasing carbon monoxide (CO) and free iron. This reaction is the major source of intracellular CO, an important physiological messenger that has been linked to multiple biological processes. Similar to nitric oxide, CO increases cGMP through activation of soluble guanylate cyclase, acts as a neurotransmitter in the brain, inhibits platelet aggregation, and decreases vascular tone (9, 29). Three isoforms of HO, products of distinct genes, have currently been identified: HO-1 (32 kDa), HO-2 (36 kDa), and HO-3 (30 kDa) (15, 16). Whereas HO-2 and HO-3 are constitutively expressed, HO-1 is inducible by a variety of stimuli (5, 16, 28).

Both in vivo and in vitro studies have shown that hypoxia increases the level of HO-1 mRNA, protein, activity, and CO production in whole lung and vascular smooth muscle cells (3, 12, 14, 20, 26).

Hypoxia-driven changes in the pulmonary vasculature are well characterized. Manifestations of hypoxia-induced pulmonary hypertension include increased pulmonary artery pressure (Ppa), vascular remodeling, and right ventricular (RV) hypertrophy (13, 17, 18). Although hypoxia induces HO-1 expression, the role of HO-1 and CO production in the development of hypoxic pulmonary hypertension remains unclear. Christou et al. (6) have reported that augmented induction of HO-1 by NiCl2 prevents the development of hypoxia-induced pulmonary hypertension and vascular remodeling in rats. Furthermore, these authors hypothesized this protection was related to CO production, which decreased pulmonary vascular tone and inhibited smooth muscle cell proliferation. In contrast, Yet et al. (30) demonstrated that hypoxia induced similar increases in RV systolic pressure and vascular remodeling in both wild-type and HO-1 null mice, suggesting that HO-1 does not play a role in the development of hypoxic pulmonary hypertension. Additionally, Carraway et al. (4) reported enhanced remodeling in rats exposed to hypoxia and CO gas, whereas Yun et al. (32) observed the opposite. Thus the role of HO and CO production in hypoxic pulmonary hypertension remains uncertain.

Against this background, we tested the hypothesis that blocking HO activity with the HO inhibitor tin protoporphyrin (SnPP) would exacerbate the development of hypoxic pulmonary hypertension in rats. Rats were injected weekly with either SnPP or saline and maintained under room air or hypobaric hypoxia for 5 wk. Rats were then catheterized, and hemodynamic measurements were made during subsequent room air and acute hypoxia exposures.

METHODS

All studies were approved by the Animal Care and Use Committee of the University of Colorado Health Sciences Center.

HO inhibition. SnPP (Porphyrin Products; Logan, UT) was solubilized in 0.1 M NaOH and diluted 1:4 in sterile saline, pH was adjusted to 7.4, and 50 μmol/kg body wt was injected subcutaneously in volume 0.2 ml. This dose and route of administration of SnPP has been shown to completely inhibit HO activity in lipopolysaccharide-treated rats, which markedly induces HO-1 without toxicity (24). In addition, other groups have reported inhibition of HO activity using smaller doses (25–10 μmol/kg) of SnPP in mice and rats (8, 25). SnPP

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solution was freshly prepared on injection days and protected from light. Control treatment consisted of injection of 0.2 ml sterile saline.

Animals. Pathogen-free male adult Sprague-Dawley rats (250 g) were used in all experiments. Pulmonary normotensive controls were kept at Denver’s altitude of 5,280 ft. Chronically hypoxic pulmonary hypertensive animals were exposed to a simulated altitude of 17,000 ft in a hypoxic chamber continuously flushed with room air to prevent accumulation of CO₂, NH₃, and water vapor. All rats were exposed to 12-h light/12-h dark cycle and allowed free access to standard rat food and water 24 h a day for 5 wk. Rats were injected weekly with either saline or SnPP (50 μmol/kg) as described above.

Conscious catheterized rats. As previously described (27), rats chronically treated with either saline or SnPP and exposed to normoxia or chronic hypoxia were anesthetized with intramuscular ketamine (100 mg/kg; Fort Dodge Laboratories; Fort Dodge, IA) and Rompun (15 mg/kg; Miles, Shawnee, KS) for placement of catheters in the pulmonary and right carotid arteries, as well as the right jugular vein. The intravascular location of the catheter tips were determined by the blood pressure tracings, and the catheters were secured, filled with heparinized saline containing 1 mg/ml chloramphenicol, sealed, and tunneled subdermally to the back of the neck where they were exteriorized and enclosed in a small plastic cap. The incisions were closed, and the rats were allowed to recover for 48 h at the altitude of Denver. After recovery, a conscious rat was placed in a ventilated clear plastic chamber flushed with either room air or 10% O₂ for measurement of pulmonary and systemic pressures (Pₚₛₛ) and cardiac output. Pulmonary and systemic pressures were measured with transducers (Statham Instruments; Hato Rey, PR), and mean pressure was calculated by computer. Cardiac output was measured by dye-dilution technique.

Hematocrit and RV hypertrophy. Immediately after the rats were euthanized, blood was collected, and the heart and lungs were removed en bloc. The heart was resected, and the atria were removed to the plane of the atrial-ventricular valves. The RV free wall was then dissected free of the LV and septum. The RV and LV plus septum were weighed, and the RV/LV + S ratio was calculated. Hematocrit was measured using a capillary tube and standard techniques.

Echocardiograms. Hypoxic rats (n = 2 saline and SnPP treated) were anesthetized with an intraperitoneal injection of ketamine and Rompun as previously described. The animal was placed in a supine position, and the abdomen and thoracic region were then shaved. With the use of a Vingmed 5 echocardiography machine with a 10-MHz probe, four standard measurements were recorded: parasternal short axis, parasternal long axis, RV diameter in diastole, and RV anterior wall thickening calculated as the percentage of thickening between RV anterior wall measurement in systole and diastole.

Data analysis. Total pulmonary resistance (TPR) was calculated by dividing mean Pₚₛₛ by cardiac output. Total systemic resistance was calculated by dividing mean Pₛₛ by cardiac output. Statistical analysis was done by Student’s t-test or ANOVA with Fisher’s post hoc test. Differences were considered significant at P < 0.05. Data are presented as means ± SE.

RESULTS

Inhibition of HO activity does not alter the development of pulmonary hypertension but rather prolongs the acute hypoxic pulmonary vasoconstriction in normoxic rats. Chronic hypoxia increased hematocrit values significantly from 21.6 ± 0.7% to 38.6 ± 2.8% (P < 0.05) in saline-treated rats and 21.8 ± 0.9% to 36.4 ± 2.6% (P < 0.05) in SnPP-treated rats. Pₚₛₛ in chronically hypoxic (CH) rats was increased compared with normoxic rats but did not differ between saline- and SnPP-treated rats within groups (Fig. 1).

To test the effect of chronic HO inhibition on pulmonary artery vasoconstrictor tone, normoxic and CH rats were challenged with acute hypoxia, 10% O₂ for a total of 30 min. Saline- and SnPP-treated normoxic rats exhibited comparable increases in Pₚₛₛ during the first 5 min of the acute hypoxic challenge. However, after 30 min of acute hypoxic exposure, SnPP-treated rats exhibited a more sustained pressor response compared with saline-treated rats (Fig. 2). As expected, CH rats had elevated Pₚₛₛ at all time points compared with normoxic rats regardless of treatment. Interestingly, CH rats treated with SnPP tended to have an acute increase in Pₚₛₛ after 5 min of hypoxic exposure more akin to normoxic rats versus salinel treated CH cohorts (Saline Norm 9.24 ± 1.26 mmHg; SnPP Norm 10.48 ± 1.41 mmHg; saline CH 3.9 ± 2.2 mmHg; and SnPP CH 8.5 ± 2.2 mmHg change in Pₚₛₛ). This initial enhanced vasoconstriction was not sustained after 30 min of hypoxic exposure and did not reach statistical significance (Fig. 2).

Effects of HO activity inhibition on Pₛₛ. To determine the effect of chronic inhibition of HO activity on the systemic circulation, we measured carotid artery pressure in conscious normoxic and CH rats during room air breathing and acute hypoxic challenge. Normoxic SnPP-treated rats had a small but significant decrease in baseline Pₛₛ compared with saline-treated rats; no significant difference was detected between saline- and SnPP-treated CH rats (Fig. 3). During the first 5 min of the acute hypoxic challenge, saline-treated CH rats experienced a pronounced dilatation compared with saline-treated normoxic rats (Fig. 4). In SnPP-treated rats, the acute hypoxia-induced decrease in Pₛₛ was not different between normoxic or CH groups.

Changes in cardiac output, total pulmonary resistance, and total systemic resistance. Baseline cardiac output during room air breathing was comparable between saline-treated normoxic and CH rats (Fig. 5). In contrast, normoxic rats treated with SnPP had an increase in baseline cardiac output, whereas SnPP-treated CH rats showed a decrease. Interestingly, this difference remained significant until 30 min after the acute hypoxic challenge. Analogous to our Pₚₛₛ results, total pulmonary resistance was elevated in CH rats, regardless of treat-
ment, at all time points compared with normoxic animals (Table 1). No differences in total pulmonary resistance were detected between saline- and SnPP-treated rats within groups. Although total systemic resistance during room air exposure was similar between all groups, total systemic resistance was lower in normoxic-SnPP rats compared with saline-treated normoxic and SnPP-treated CH rats after 5 min of hypoxic exposure (10% O₂) (Table 2). Moreover, total systemic resistance remained lower after 30 min of hypoxic challenge in normoxic SnPP-treated rats compared with CH SnPP-treated rats.

Inhibition of HO activity attenuates hypoxia-induced RV hypertrophy. Consistent with \( P_{pa} \) measurements, all CH rats had increased RV mass compared with normoxic rats (Fig. 6). However, treatment with SnPP reduced RV mass in both normoxic and CH groups compared with saline-treated rats. No difference was detected in LV per body weight among the groups (Saline Normoxic = 2.14 ± 0.089 mg/g, SnPP Normoxic = 1.936 ± 0.066 mg/g, Saline CH = 2.035 ± 0.064 mg/g, and SnPP CH = 2.042 ± 0.083 mg/g). In addition, RV-to-body weights measurements correlated with RV/LV + S data, suggesting the variation in cardiac mass was selective for the RV (data not shown).

**RV dilatation and decreased RV function in SnPP-treated rats.** To determine whether chronic HO inhibition altered RV function and size, echocardiograms were performed in a limited number of CH SnPP- and saline-treated rats (\( n = 2 \) in each group). SnPP treatment was associated with RV dilatation and decreased RV function (measured as %fractional shortening) (Fig. 7).

**DISCUSSION**

We found that endogenous HO does not normally play a significant role in modulating the development of pulmonary
HO IN HYPOXIA-INDUCED RV HYPERTROPHY AND VASOREACTIVITY

hypertension in rats under CH conditions but may be involved in regulation of pulmonary and systemic vasomotor tone following chronic hypoxia. In this study, blocking HO activity with SnPP had no effect on either baseline or hypoxia-induced elevation in Ppa. Chronic inhibition of HO resulted in more rapid systemic vasodilation to acute hypoxia in normoxic rats. Our data further suggest HO activity may be critical for the normal adaptation of the RV to pressure overload during hypoxia. In the present study, we found that SnPP treatment in Sprague-Dawley rats decreased hypoxia-induced RV hypertrophy, impaired RV function, and decreased cardiac output.

Previous investigations have shown augmentation of endogenous HO-1 can protect rats exposed to hypoxia from developing pulmonary hypertension, although genetic deletion of HO-1 did not exacerbate elevation of RV pressure or severity of vascular remodeling in mice exposed to chronic hypoxia (6, 30). More recently transgenic mice generated to target HO-1 overexpression to the lung were protected against the development of pulmonary inflammation, pulmonary hypertension, and vascular remodeling induced by hypoxia (19). Normally in rat lungs, HO-1 protein and activity levels are increased only during the first few days of CH exposure, even though persistent elevation of carboxyhemoglobin levels suggest CO production at nonpulmonary sites (3). Recently, two reports from the same group found enhanced hypoxia-induced pulmonary hypertension and vascular remodeling in intermittently hypoxic (6 h per day) rats following daily treatment with zinc protoporphyrin (ZnPP) (10, 32). Taken together, these studies suggest that a sustained induction of HO-1 is necessary to protect against hypoxia-induced pulmonary consequences. In contrast to these reports, our results suggest that endogenous HO does not normally play a significant role in attenuating the rapid systemic vasodilation to acute hypoxia in normoxic rats.

In this study, blocking HO activity with SnPP had no effect on either baseline or hypoxia-induced elevation of Ppa, supporting earlier observations that deletion of HO-1 in mice had little effect on RV systolic pressure and pulmonary vascular remodeling after prolonged hypoxic exposure (30). It was previously speculated the CO released by HO-2 might be

Table 1. Total pulmonary resistance

<table>
<thead>
<tr>
<th>Group</th>
<th>Room Air</th>
<th>5 min</th>
<th>30 min</th>
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<tbody>
<tr>
<td>Normoxic Saline</td>
<td>158±15</td>
<td>244±12</td>
<td>206±20</td>
</tr>
<tr>
<td>SnPP</td>
<td>150±24</td>
<td>222±25</td>
<td>234±29</td>
</tr>
<tr>
<td>Chronically hypoxic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>305±36†</td>
<td>378±59†</td>
<td>388±54†</td>
</tr>
<tr>
<td>SnPP</td>
<td>311±39†</td>
<td>427±62†</td>
<td>454±62†</td>
</tr>
</tbody>
</table>

Values are means ± SE (in mmHg·1−1·min). *Compared with SnPP normoxic; room air, P < 0.004; hypoxia 5 min, P < 0.04; hypoxia 30 min, P < 0.03. †Compared with SnPP normoxic; room air, P < 0.002; hypoxia 5 min, P < 0.05; hypoxia 30 min, P < 0.007.

Table 2. Total systemic resistance

<table>
<thead>
<tr>
<th>Group</th>
<th>Room Air</th>
<th>5 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic Saline</td>
<td>912±68</td>
<td>972±58</td>
<td>894±68</td>
</tr>
<tr>
<td>SnPP</td>
<td>813±122</td>
<td>751±80†</td>
<td>817±88†</td>
</tr>
<tr>
<td>Chronically hypoxic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Saline</td>
<td>966±63</td>
<td>875±66</td>
<td>947±65</td>
</tr>
<tr>
<td>SnPP</td>
<td>1,007±67</td>
<td>1,040±66</td>
<td>1,077±82</td>
</tr>
</tbody>
</table>

Values are means ± SE (in mmHg·1−1·min). *Compared with Saline normoxic; hypoxia 5 min, P < 0.04. †Compared with SnPP CH; hypoxia 5 min, P < 0.01; hypoxia 30 min, P < 0.05.
sufficient to prevent the expected exacerbation of pulmonary hypertension and vascular remodeling in null mice exposed to hypoxia. Because SnPP is a nonselective HO inhibitor, and in the dose given completely abolished lung HO activity in LPS-treated rats (24), our data suggest that neither HO-2 nor HO-3 moderate the development of hypoxia-induced pulmonary hypertension in HO-1 null mice.

These results are in contrast to the reports of Gong et al. (10) and Yun et al. (32) who found augmented pulmonary hypertension in intermittent hypoxia when HO was inhibited by ZnPP, a selective inhibitor of HO-1. A major difference between these and our studies is the type and duration of hypoxic exposure. In our studies, rats were exposed to hypoxia 24 h per day for 5 wk, whereas in those of Gong and Yun, exposure was 6 h per day for 14 days. Intermittent hypoxia may cause pulmonary vascular changes due to different mechanisms than chronic hypoxia (2). Additionally, hypobaric exposure (in our study) and hypoxic gas (in studies of Gong and Yun) exposure may also contribute to these differences (i.e., ammonia, CO2 levels). Also, different strains of rats were used in our study (Sprague-Dawley) compared with those used in the study by Gong and Yun (Wistar). Previous reports have suggested that Wistar rats develop more marked RV hypertrophy than Sprague-Dawley rats following hypoxia (1). In the present study, we found that SnPP treatment in Sprague-Dawley rats decreased hypoxia-induced RV hypertrophy, impaired RV function, and decreased cardiac output. One possibility is that HO activity may play a more important protective role in hearts of Sprague-Dawley, compared with Wistar, rats such that loss of HO activity impairs hypoxia-induced remodeling and ventricular performance. Another difference was the use of HO inhibitors. ZnPP may have more nonspecific, non-HO inhibiting effects than SnPP on regulation of vascular tone (22).

On the other hand, our results do suggest that in chronic hypoxia, HO activity may be important for the adaptation of vasomotor tone to acute hypoxia. It has been previously reported that acute hypoxic pulmonary vasoconstriction is enhanced in normoxic rats when HO is acutely inhibited (33). Therefore, in our study, we wanted to identify the effects on vasomotor tone following chronic inhibition of HO activity and to determine whether the response would be modulated by chronic hypoxia. SnPP-treated normoxic rats had a more sustained pulmonary vascular pressor response compared with saline-treated normoxic rats, suggesting that HO activity may be involved with compensatory vasodilatation associated with acute hypoxic pulmonary vasoconstriction (Fig. 2). This is in agreement with enhancement of hypoxic pulmonary vasoconstriction following acute HO inhibition (33). This pattern was not seen in the CH rats, suggesting that following CH, HO activity does not play a significant role in enhanced acute hypoxic pulmonary vasoconstriction. Similarly to the pulmonary vasculature, chronic inhibition of HO activity also altered
The systemic vascular response to acute hypoxia. Whereas all groups had comparable systemic arterial pressures after 30 min of acute hypoxic exposure, chronic inhibition of HO resulted in more rapid systemic vasodilation in normoxic rats. These data suggest HO activity may play a role in regulating systemic vasomotor tone to hypoxia as well as baseline systemic blood pressure under normoxic conditions (Figs. 3 and 4). Previous in vitro studies have identified the role of increased endogenous CO in the blunted vasoreactivity to phenylephrine in systemic arterial rings following CH (11, 23).

One of the hallmarks of hypoxia-induced pulmonary hypertension is the development of RV hypertrophy as the heart adapts to increased blood viscosity and pulmonary vascular resistance. Surprisingly, SnPP-treated animals from both normoxic and CH groups showed a reduction in RV mass. A recent study by Nagaoa et al. (21) demonstrated that mild hypoxia can lead to slight increases in both mean Ppa and RV hypertrophy in rats. Therefore, the decrease in RV mass in the SnPP-treated normoxic group might be ascribed to the disruption of the mild RV hypertrophy that develops in animals maintained at the altitude of Denver. Accumulating evidence confirms that HO-1 is induced in the heart in response to other stimuli such as hypoxia and increased Ppa, suggesting that HO activity may play a protective role in cardiac tissue undergoing stress. For example, cardiac-specific expression of HO-1 was recently demonstrated to provide protection against ischemia and reperfusion injury in transgenic mice (31). Furthermore, HO-1 knockout mice exposed to hypoxia for 5–7 wk develop severe RV dilatation with increased mural thrombi and cardiomyocyte apoptosis compared with wild-type mice (30). These data support our echocardiogram findings of increased RV dilatation and decreased RV function in SnPP-treated rats compared with saline-treated CH rats. In contrast to our results, Yet et al. (30) noted an exaggerated increase in total ventricular mass in chronically hypoxic HO-1 knockout mice compared with wild-type mice. Gong and Yan (10, 32) also reported increased RV hypertrophy in ZnPP-treated intermittently hypoxic rats. Given that in vitro studies have demonstrated mouse cardiac myocytes exhibit a unique autonomous hypertrophy response compared with rat neonatal myocytes, and that Wistar rats have a more vigorous RV hypertrophy response than Sprague-Dawley rats, it is possible that the variance between strains and species could account for the discrepancy between these studies and our data (7). Another explanation for the disparity between our study and Yet et al. (30, 31) is that HO-1 null mice retain the ability to generate a compensatory increase in either HO-2 and/or HO-3 activity, whereas our use of SnPP may inhibit all HO activity, which may have mediated compensatory increases in other HO isoforms unlikely (24).

In summary, we demonstrate that HO activity does not alter the development of hypoxia-induced pulmonary hypertension but rather plays an adaptive role in pulmonary and systemic vasoreactivity during acute hypoxic challenge. More importantly, HO-1 may play a cardioprotective role in promoting RV hypertrophy and preserving RV function in hypoxia. Cardiac muscle failure is one of the most important problems in cardiovascular medicine, and understanding how HO conditions cardiac tissue during hypoxic stress may lead to novel strategies for treating heart disease.

REFERENCES