Kidney-Specific Induction of Heme Oxygenase-1 Prevents Angiotensin II Hypertension

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Abstract—The main goal of this study was to determine whether kidney-specific induction of heme oxygenase-1 (HO-1) can prevent the development of angiotensin (Ang) II–dependent hypertension. To test this hypothesis, intrarenal medullary interstitial catheters were implanted into the left kidney of uninephrectomized mice. Infusion of cobalt protoporphyrin (CoPP; 250 μg/mL; at 50 μL/h for 48 hours) resulted in significant induction of HO-1 in the renal medulla when examined 2 weeks after the infusion with no induction observed in other organs, such as the heart or liver. Next, we examined the effect of renal-specific induction of HO-1 on the development of Ang II–dependent hypertension. CoPP or vehicle (0.1 mol/L NaOH [pH 8.3]) was infused as indicated above 2 days before implantation of an osmotic minipump, which delivered Ang II or saline vehicle at a rate of 1 μg/kg per minute. Mean arterial pressure was measured in conscious, unrestrained mice for 3 consecutive days starting on day 7 after implantation of the minipumps. Mean arterial pressure averaged 114±5, 122±4, 162±2, and 125±6 mm Hg in vehicle-, intrarenal medullary interstitial CoPP–, Ang II–, and Ang II + intrarenal medullary interstitial CoPP–treated mice, respectively (n=6 or 7). These results demonstrate that kidney-specific induction of HO-1 prevents the development of Ang II–dependent hypertension and that induction of HO-1 in the kidney may be the mechanism by which systemic delivery of CoPP lowers blood pressure in Ang II–dependent hypertension. (Hypertension. 2008;52:660-665.)

Key Words: heme oxygenase-1 ■ kidney ■ angiotensin II ■ hypertension ■ cobalt protoporphyrin

Heme oxygenase-1 (HO-1) is an important endogenous cellular defense protein that is induced by a wide variety of stimuli, including metals, toxins, and hypoxia. Several studies have demonstrated that induction of HO-1 can prevent the development of experimental hypertension. Chemical induction of HO-1 with hemin or cobalt protoporphyrin (CoPP) has been reported to attenuate hypertension in the spontaneously hypertensive rat, as well as in angiotensin II (Ang II)–dependent hypertension in mice. Genetic alteration of HO-1 has also been reported to influence blood pressure. Lentiviral overexpression of HO-1 in the rat prevents hypertension in both the spontaneously hypertensive rat and Ang II–dependent models. In contrast, mice lacking HO-1 exhibit an increase in 1-kidney, 1-clip renovascular hypertension as compared with wild-type mice.

Despite the strong evidence for a role of the HO system in the regulation of blood pressure, it has been difficult to determine the mechanism of the blood pressure–lowering action of HO-1 induction. This has been mainly because of the fact that previous studies have relied on systemic induction of HO-1 to lower blood pressure, so the role of specific organs, such as the kidney, in this response is not clear. There are several possible mechanisms by which systemic induction of HO-1 may lower blood pressure in hypertension, including improvement of vasodilator function, reduction of sympathetic outflow, and increases in natriuresis.

In the kidney, HO has important actions in both the vasculature and tubules, where it is an important regulator of medullary blood flow and sodium excretion. Recent studies have demonstrated an increase in renal medullary HO activity in response to increases in renal perfusion pressure and an important role of renal medullary HO in adaptation to a high-salt diet. Additional studies from our laboratory have demonstrated that systemic induction of HO-1 in Ang II–hypertensive mice is associated with a decrease in superoxide production in the renal medulla. However, the importance of this finding to the blood pressure–lowering actions of systemic HO-1 induction could not be addressed, because HO-1 was increased throughout the cardiovascular system. The goal of the present study was to determine whether kidney-specific induction of HO-1 could attenuate Ang II–dependent hypertension. This was accomplished by specifically inducing HO-1 in the kidney via intrarenal medullary interstitial (IRMI) infusion of CoPP.
To specifically increase HO-1 levels in the kidney, CoPP was administered via intramedullary interstitial catheters implanted via intramedullary interstitial catheters into the left kidney. After 3 days, IRMI infusions were switched to saline to either CoPP or vehicle for 2 days and then stopped. Osmotic minipumps containing Ang II were then implanted 2 days later. After 5 days, carotid artery catheters were implanted and blood pressures were recorded for 3 consecutive days after a 2-day recovery period.

Methods

Animals
Experiments were performed on 12- to 16-week-old male C57BL/6J mice obtained from Jackson Laboratories (Bar Harbor, Maine). Mice were fed a standard diet containing 0.29% NaCl and were provided water ad libitum. All of the animal protocols were approved by the institutional animal care and use committee at the University of Mississippi Medical Center. In all of the mice, the right kidney was removed, and the mice were allowed 5 days of recovery. After this time, intramedullary interstitial catheters were then implanted into the remaining kidney, and saline was infused through the catheter for a period of 3 days. Intramedullary interstitial catheters were modified as described previously for the rat14,15 and implanted 1.5 to 2.0 mm into the left kidney. After 3 days, IRMI infusions were switched to either CoPP (250 µg/mL; at 50 µL/h) or vehicle (0.1 NaOH [pH 8.0]) for 2 days and the infusion stopped. After 2 days, mice were implanted with osmotic minipumps, which infused Ang II or vehicle (saline) at a rate of 1 µg/kg per minute. Five days later, carotid artery catheters were implanted and blood pressure was measured in conscious, unrestrained mice for 3 consecutive days after a 2-day recovery period. The experimental protocol is outlined in Figure 1.

Heme Oxygenase Assay and Western Blots
Heme oxygenase assays and Western blots were performed on tissue lysates taken from mice at the end of the experimental protocol as described previously.4 An extinction coefficient of 40 mmol/L per cm for bilirubin was used.

Measurement of Superoxide Production
Superoxide production was measured using the lucigenin technique as described previously.4,16,17

Heme Content
Heme content in the medulla was measured spectrophotometrically by the absorbance of each sample at 388, 450, and 330 nm using the correction formula $A_388 = 2A_{450} - (A_{450} + A_{330})$, as described previously.4,18 The amount of heme in each sample was then normalized to the total protein and expressed as nanomoles of heme per milligram of protein.

Statistics
Mean values±SEs are presented. Significant differences between mean values were determined with the use of an ANOVA followed by a posthoc test (Dunnet's). A P<0.05 was considered to be significant.

Results

Kidney-Specific Induction of HO-1 With IRMI Infusion of CoPP
To specifically increase HO-1 levels in the kidney, CoPP was administered via intramedullary interstitial catheters implanted into the renal medulla of the mouse kidney. This approach has been reported previously to result in kidney-restricted delivery of compounds.15,19 CoPP was administered into the renal medulla for a period of 48 hours, and then the infusion stopped. Two weeks later, the levels of HO-1 and HO-2 were examined in the cortex and medulla of the kidney, as well as in the liver and the heart. IRMI infusion of CoPP resulted in the specific induction of HO-1 in the renal medulla with no evidence for induction in extrarenal tissues (Figure 2). The time course of induction of HO-1 with CoPP is consistent with previous reports of the effect of CoPP on HO-1 protein.20 IRMI infusion of CoPP did not have any effect on the levels of HO-2 protein in any tissue examined.

IRMI Infusion of CoPP Attenuates Ang II–Dependent Hypertension
Mean arterial pressure (MAP) averaged 114±5 mm Hg in vehicle mice and was slightly higher (122±4 mm Hg) in mice that received IRMI infusion of CoPP. MAP was significantly increased to 162±2 mm Hg in Ang II–treated mice that received IRMI infusion of vehicle (Figure 3). MAP was significantly attenuated in Ang II–treated mice that received...
IRMI infusion of CoPP and averaged 125±6 mm Hg (Figure 3). Heart rate was similar in all of the groups, averaging 639±26, 564±21, 598±46, and 567±20 bpm in control, IRMI CoPP, Ang II, and Ang II+IRMI CoPP mice, respectively.

**IRMI Infusion of CoPP Increases HO-1 Protein and HO Activity in the Renal Medulla**
IRMI infusion of CoPP significantly increased HO-1 protein levels in the medulla of both control mice and mice treated with Ang II (Figure 4A). Treatment with Ang II alone did not have any significant effect on the levels of HO-1 protein in the renal medulla (Figure 4A). IRMI infusion of CoPP did not have any effect on the levels of HO-2 protein in the renal medulla (Figure 4A). IRMI infusion of CoPP did not have any significant effect on the levels of HO-1 protein in the renal medulla (Figure 4A). IRMI infusion of CoPP did not have any significant effect on the levels of HO-1 protein in the kidneys of IRMI CoPP– and IRMI CoPP–infused mice receiving IRMI CoPP, averaging 179±5 pmol of bilirubin/mg per hour compared with both vehicle and IRMI vehicle+Ang II mice (Figure 4B; P<0.05). IRMI infusion of CoPP had no significant effect on renal cortical HO activity (Figure 4C).

**IRMI Infusion of CoPP Decreases Ang II–Mediated Increases in Heme Content and Superoxide Production**
Heme is a pro-oxidant that is capable of producing reactive oxygen species and has been reported as a source of peroxynitrite, which could contribute to increases in protein nitrosylation.21 Consistent with previous reports,4,22 Ang II infusion increased medullary heme content from 1.5±0.2 to 3.0±0.4 nmol/mg of protein compared with control mice (Figure 5). IRMI CoPP treatment alone had no significant effect on medullary heme content and averaged 1.2±0.2 nmol/mg of protein. IRMI CoPP treatment significantly lowered medullary heme content in Ang II–infused mice to levels similar to those observed in control mice averaging 1.2±0.2 nmol/mg of protein (Figure 5).

Superoxide production as measured with lucigenin chemiluminescence was significantly increased as compared with
control by both IRMI infusion of CoPP, as well as Ang II infusion (630 ± 121 versus 1078 ± 164 versus 1500 ± 207 relative light units per minute per milligram of protein; Figure 6). Previous IRMI infusion of CoPP in Ang II–infused mice resulted in a significant decrease in superoxide production toward control levels averaging 778 ± 193 relative light units per minute per milligram of protein (Figure 6). This effect of previous CoPP treatment on Ang II–mediated superoxide production in the renal medulla was consistent with previous observations with systemic administration of CoPP.4

IRMI infusion of CoPP by itself significantly decreased catalase and extracellular superoxide dismutase (EC-SOD) levels in the renal medulla (Figure 7). Ang II treatment was also associated with a significant decrease in renal medullary catalase protein, which was not normalized by IRMI infusion of CoPP. Ang II treatment also resulted in a significant increase in medullary EC-SOD levels, which was consistent with our previous results; however, IRMI of CoPP was not able to lower EC-SOD levels in Ang II–infused mice (Figure 7). No changes in the levels of CuZn or Mn SOD protein in the medulla were observed with any of the experimental treatments (Figure 7).

Figure 5. Renal medullary heme content in control, IRMI CoPP−, Ang II−, and IRMI CoPP+Ang II–treated mice. Medullary heme content was increased in Ang II–treated mice, and this increase was normalized with IRMI CoPP infusion. *Significant difference from control (P<0.05).

Figure 6. Renal medullary superoxide production in control, IRMI CoPP−, Ang II−, and IRMI CoPP+Ang II–treated mice. Medullary superoxide production was increased in Ang II–treated mice, and this increase was normalized with IRMI CoPP infusion. *Significant difference from control (P<0.05). †Significant difference from Ang II (P<0.05).

Discussion

There is considerable evidence to support the antihypertensive actions of systemic HO-1 induction. Studies have consistently demonstrated that whole-body induction of HO-1, either chemically or genetically, can lower blood pressure in several different models of hypertension.2–4 We report the first evidence that kidney-specific induction of HO-1 prevents the development of hypertension. We used chronic IRMI infusions of CoPP to specifically increase HO-1 in the kidney of the mouse without affecting the levels of HO-1 in extrarenal tissues, such as the liver and heart. This approach allowed us to specifically prove that exclusive induction of HO-1 in the kidney prevents Ang II–dependent hypertension.

IRMI infusion of CoPP specifically increases HO-1 protein and activity in the medulla and has a small effect on cortical HO protein and activity. These results indicate that specific increases in medullary HO-1 mediate the antihypertensive actions of IRMI infusion of CoPP. Previous studies in the rat have demonstrated that overexpression of HO-1 in the thick ascending loop of Henle can protect against Ang II–mediated oxidative injury.23 In the present study, we did not distinguish between outer or inner medullary induction of HO-1 with IRMI infusion of CoPP. Thus, it is unknown what specific cell type or types may be mediating the antihypertensive actions of IRMI infusion of CoPP. Future studies using transgenic models in which HO-1 can be induced in specific cell types within the renal medulla will allow us to answer this question.

There are several mechanisms by which increases in medullary HO activity can prevent Ang II hypertension. HO-derived CO has been demonstrated previously to protect the renal vasculature against excessive vasoconstriction from pressor agents such as Ang II.24 Inhibition of HO activity results in a marked decrease in renal medullary blood flow, which could result in the impairment of pressure natriuresis.12 Therefore, it is possible that induction of HO-1 in the medulla with IRMI infusion of CoPP leads to increases in vascular CO production, which act to preserve renal medullary blood flow, and enhances pressure-natriuresis during chronic increases in Ang II. Additional, more detailed studies specifically examining regional blood flow in the kidney will be required to examine this possibility.

An increase in renal medullary superoxide production can be prohypertensive through vascular and tubular mechanisms. Recent studies by Mori and Cowley25 have demonstrated that Ang II–stimulated increases in superoxide generation can influence tubulovascular cross-talk and reduce the levels of NO leading to decreases in renal medullary blood flow.25 Similarly, studies have also demonstrated that increased superoxide production in renal tubules can increase sodium reabsorption in the thick ascending loop of Henle directly or through decreases in the bioavailability of NO.26,27 In agreement with previous results,4 Ang II infusion in the present study caused a significant increase in renal medullary heme content. Increases in heme levels can result in increased generation of oxidants and have detrimental affects on both vascular and tubular function.22 The increase heme content in the renal medulla was completely normalized by IRMI infusion of CoPP. Likewise, Ang II infusion also resulted in
an increase in the levels of superoxide anion in the renal medulla. This increase in superoxide production in the medulla was normalized by IRMI infusion of CoPP. However, unlike systemic administration of CoPP, IRMI infusion of CoPP alone resulted in an increase in medullary superoxide production. IRMI infusion of CoPP alone also resulted in decreased levels of catalase, as well as EC-SOD, both of which may result in loss of antioxidant capacity and account for the increase in medullary superoxide levels observed in mice receiving IRMI infusion of CoPP. This significant increase in superoxide generation may also be responsible for the slightly higher blood pressure observed in mice infused with IRMI CoPP alone. Despite increasing renal medullary superoxide levels when infused alone, treatment with CoPP normalized the levels of superoxide in the renal medulla of Ang II–infused mice. The explanation for the decrease in superoxide production in Ang II–infused mice treated with CoPP is not clear but is likely because of the direct effects of increases in bilirubin or CO to inhibit reduced nicotinamide-adenine dinucleotide phosphate oxidase.28,29

Another possible mechanism for the antihypertensive effects of specific HO-1 induction in the kidney could be direct effects on renal tubules. A recent study in rats reported enhancement of CO production in response to increases in renal perfusion pressure.13 Moreover, inhibition of HO activity in the medulla resulted in significantly blunted pressure natriuresis and salt-sensitive hypertension.13 These observations are consistent with earlier reports that induction of HO with heme results in inhibition of tubular reabsorption of sodium and water.11 We did not observe any increase in HO activity in the medulla of the mouse in response to chronic Ang II infusion despite increases in renal perfusion pressure and previous reports of stimulation of HO activity by Ang II in the rat kidney.13,30 The lack of induction of HO-1 in response to Ang II infusion is consistent with our previous studies examining the renal regulation of Ang II by HO-14 and may be because of differences in the regulation of HO-1 in the kidney of mice and rats.

**Perspectives**

Our results demonstrate that kidney-specific induction of HO-1 has a powerful antihypertensive action during chronic Ang II hypertension. These observations suggest the possibility of developing antihypertensive therapies that exclusively target HO-1 in the kidney to avoid some of the potentially deleterious actions of systemic HO-1 induction. Our results also demonstrate that decreases in both renal medullary heme content and superoxide production are associated with the reduction in blood pressure observed with kidney-specific induction of HO-1. It is possible that HO-1 decreases in renal medullary reactive oxygen species may mediate the antihypertensive action of HO-1 induction in Ang II hypertension. Additional studies are required to determine whether the antioxidant actions of HO-1 induction in the kidney act through vascular or tubular mechanisms to lower blood pressure in Ang II hypertension.

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Disclosures
None.

References
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