

# Heme Oxygenase-1 Is Upregulated in the Kidney of Angiotensin II–Induced Hypertensive Rats

## Possible Role in Renoprotection

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**Abstract**—In this study, we investigated the regulation and physiological role of heme oxygenase-1 (HO-1) in the kidney of rats with hypertension. Rats were continuously administered either angiotensin II (Ang II) or norepinephrine with an osmotic minipump for up to 7 days. Ang II infusion decreased the glomerular filtration rate (GFR) as determined through creatinine clearance ( $3.2 \pm 0.2$  versus  $1.2 \pm 0.2$  mL/min with Ang II infusion,  $P < 0.01$ ) and increased proteinuria ( $9.7 \pm 1.3$  versus  $28.1 \pm 7.2$  mg/d with Ang II infusion,  $P < 0.01$ ). In contrast, norepinephrine did not alter these laboratory values. Ang II infusion significantly increased HO-1 expression in mRNA ( $442 \pm 98\%$  of control at day 5,  $P < 0.01$ ) and protein levels ( $314 \pm 49\%$  of control at day 5,  $P < 0.01$ ). Immunohistochemistry showed that in the kidney of normotensive rats, HO-1 was expressed mainly in the basal side in the renal tubules. After Ang II infusion, HO-1 staining was more extensively dispersed in the tubular epithelial cells. The intraperitoneal administration of zinc protoporphyrin, an HO inhibitor, to Ang II-infused rats further decreased GFR ( $0.8 \pm 0.1$  mL/min) and increased proteinuria ( $52.5 \pm 13.0$  mg/d). In contrast, the administration of hemin, an HO inducer, ameliorated the Ang II–induced decrease in GFR ( $2.4 \pm 0.2$  mL/min) and increase in proteinuria ( $9.3 \pm 4.5$  mg/d). These data suggest that HO-1 upregulation in the kidney of Ang II–induced hypertensive rats may exert a renoprotective effect against Ang II–induced renal injury. (*Hypertension*. 2000;35:800-806.)

**Key Words:** hypertension ■ angiotensin II ■ proteinuria ■ oxidative stress ■ kidney

Hypertension induces structural and functional alterations in the kidney, eventually leading to end-stage renal disease. The control of blood pressure retards the progression of renal failure and reduces the morbidity and mortality rates associated with hypertensive vascular disease.<sup>1,2</sup> Although several clinical trials have shown a similar slowing of the progression with the use of either ACE inhibitors or other antihypertensive drugs,<sup>3</sup> the superiority of ACE inhibitors has been demonstrated in some studies. Compared with other classes of antihypertensive drugs, ACE inhibitors were more effective than other agents in preservation of the glomerular filtration rate<sup>4</sup> and in their antiproteinuric effects<sup>5,6</sup> in essential hypertension. The extra effectiveness of ACE inhibitors may derive from their ability to preferentially dilate the efferent arterioles<sup>7</sup> and their antiproliferative effects.<sup>8</sup>

Heme oxygenase (HO) is a rate-limiting enzyme of heme catabolism that has 2 isoforms: HO-1, an inducible form,<sup>9,10</sup> and HO-2, a constitutive form. Induced HO-1 is thought to act as an antioxidative and anti-inflammatory defense mechanism through the degradation of cellular heme (pro-oxidant)

and increase in biliverdin (antioxidant<sup>11</sup>). The carbon monoxide (CO) that is produced also has physiological functions, such as vascular relaxation<sup>12</sup> and inhibition of platelet aggregation,<sup>13</sup> through the activation of soluble guanylyl cyclase. The recent findings that HO-1 gene transfer ameliorated oxidative tissue injury<sup>14</sup> and that oxidant-induced cellular injury was increased in HO-1 knockout mice<sup>15</sup> and in human HO-1 deficiency<sup>16</sup> provide further direct evidence that HO-1 acts favorably against oxidative stress. In the kidney, both HO-1 and HO-2 are present in the tubular epithelial cells,<sup>17,18</sup> suggesting that the HO system also plays a role in the kidney. In fact, HO-1 induction exerts a protective effect on renal function in animal models of rhabdomyolysis,<sup>19</sup> cisplatin nephrotoxicity,<sup>20</sup> and nephrotoxic nephritis.<sup>21</sup> In previous reports, we demonstrated that HO-1 expression was regulated by Ang II in vascular smooth muscle in both pressor-dependent<sup>22</sup> and pressor-independent<sup>23</sup> manners.

Renal damage occurs via pressor-dependent and -independent mechanisms. The present study was designed to examine both such mechanisms in the angiotensin II (Ang II)

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model of hypertension in the rat. We first assessed the pressor-dependent and -independent effects of Ang II on renal function. We next examined the effect of Ang II on renal HO-1 expression. We also used immortalized rat proximal tubule cells (IRPTCs) to investigate the effect of Ang II on HO-1 expression in the proximal tubule in vitro.

## Methods

### Animal Models

The rat Ang II hypertension model was induced in Sprague-Dawley rats (Nippon Bio-Supply Center) through the subcutaneous implantation of an osmotic minipump (model 2001; Alza) as described previously.<sup>22</sup> Val<sup>5</sup>-Ang II (Sigma Chemical Co) was infused at a rate of  $0.7 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for up to 7 days. Systolic and diastolic blood pressures and heart rate were measured in conscious rats with tail-cuff plethysmography (UR-5000; Ueda Seisakusyo). In some experiments, the selective Ang II type 1 (AT<sub>1</sub>) receptor antagonist losartan ( $25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ; a kind gift from Dr R.D. Smith, Dupont/Merck) or the nonspecific vasodilator hydralazine ( $15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ; Sigma Chemical Co) was administered in the drinking water, beginning 2 days before pump implantation and throughout the Ang II infusion. So we could examine another model of hypertension, norepinephrine (NE) was infused at a rate of  $2.8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  with the same system through polyethylene tubing (Becton Dickinson) that was placed in the superior vena cava via the left external jugular vein.<sup>22</sup>

Some rats were subjected to a daily intraperitoneal injection of the HO-1 inducer hemin ( $50 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ; Sigma Chemical Co) or the HO-1 inhibitor zinc-protoporphyrin (ZnPP) ( $50 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ; Porphyrin Products), which was started 2 days before pump implantation and continued until sacrifice. In addition, some rats were subjected to a daily intraperitoneal injection of the same amount of hemin or ZnPP for 9 consecutive days without Ang II infusion.

### Assay of Plasma and Urine Samples

A 24-hour urine sample was collected before sacrifice. Plasma and urine creatinine concentrations were measured with the Jaffe reaction, and urinary protein was measured with the pyrogallol red-molybdate protein dye-binding method (SRL).

### Culture of Transformed Rat Proximal Tubular Cells

Culture of established IRPTCs was performed as described previously.<sup>24</sup> Briefly, cells were cultured in DMEM with 5% FCS. Cultures were supplemented with  $3.8 \text{ mg/mL NaHCO}_3$ ,  $25 \text{ mmol/L HEPES}$  buffer (pH 7.5),  $0.1 \text{ mmol/L sodium pyruvate}$ ,  $100 \text{ U/mL penicillin}$ ,  $100 \mu\text{g/mL streptomycin}$ , and  $0.01 \text{ mmol/L nonessential amino acids}$ . For the Ang II stimulation, cells were cultured in DMEM supplemented with 0.1% FCS for 48 hours before and throughout the stimulation.

### RNA Isolation and Northern Blot Analysis

Total RNA was isolated from homogenized heart according to the acid guanidinium thiocyanate-phenol-chloroform method with Iso-gen (Wako Pure Chemicals). Rat HO-1 cDNA (a kind gift from Dr S. Shibahara, Tohoku [Japan] University School of Medicine) was labeled with  $\alpha$ -<sup>32</sup>P-dCTP (DuPont-New England Nuclear) with commercial kits (Nippon Gene). Hybridization was performed as described previously.<sup>23</sup> Hybridized bands were visualized and quantified with a bioimaging analyzer (BAS 2000; Fuji Photo Film), and band density was normalized to the intensity of ethidium bromide-stained 28S and 18S ribosomal RNAs.

### Protein Purification and Western Blot Analysis

Protein was isolated through homogenization of samples in the lysis buffer ( $50 \text{ mmol/L HEPES}$ ,  $5 \text{ mmol/L EDTA}$ , and  $50 \text{ mmol/L NaCl}$ , pH 7.5) containing protease inhibitors ( $10 \mu\text{g/mL aprotinin}$ ,  $1 \text{ mmol/L PMSF}$ , and  $10 \mu\text{g/mL leupeptin}$ ). Antibodies against rat

**TABLE 1. Hemodynamic Parameters in Ang- and NE-infused rats**

	Systolic Blood Pressure, mm Hg	Diastolic Blood Pressure, mm Hg	Heart Rate, bpm
Control	131±3	90±4	337±15
Ang II			
1 day	153±4*	109±3*	366±13*
3 days	174±3†	120±6†	391±10†
5 days	191±3†	130±9†	403±11†
7 days	192±4†	134±14†	420±29†
NE			
1 day	168±10*	113±6*	385±18*
3 days	173±6†	119±4†	398±1†
5 days	186±10†	132±7†	407±8†
7 days	196±6†	137±5†	421±19†
Ang II+hydralazine‡	133±8	85±7	408±14†
Ang II+losartan‡	126±5	86±5	352±6
Ang II (0.25 mg · kg <sup>-1</sup> · d <sup>-1</sup> )	134±3	100±8	342±28

\* $P < 0.05$  and † $P < 0.01$  compared with the control, respectively.

Hemodynamic values were measured 7 days after Ang II infusion.

HO-1 and rat HO-2 (StressGen) were used at a 1:1000 dilution, and horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch) was used at a 1:2000 dilution. The ECL Western blotting system (Amersham Life Sciences) was used for detection. Bands were visualized with a luminoanalyzer (LAS-1000; Fuji Photo Film). Band intensity was calculated with Image software (NIH, Research Service Branch) and expressed as a percentage of control.

### Immunohistochemistry

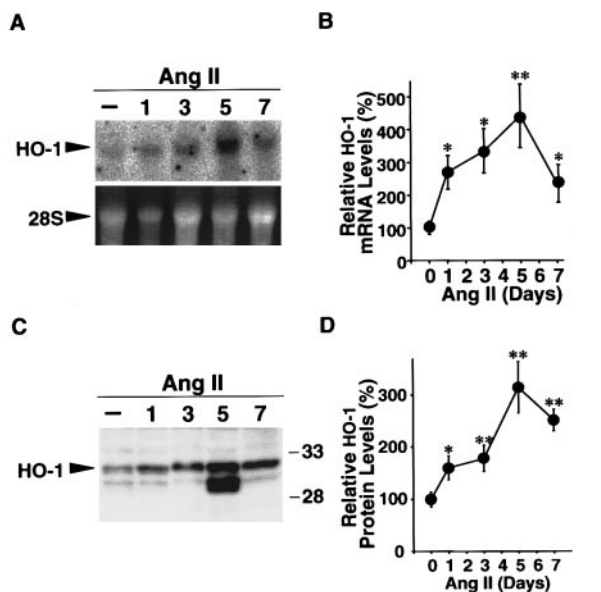
Immunohistochemistry of HO-1 was performed as described previously.<sup>25</sup> Briefly, deparaffinized sections were preincubated with 10% horse serum. Sections were then incubated with the anti-HO-1 antibody at a 1:200 dilution at 37°C for 1 hour. Slides were washed and incubated with biotinylated secondary antibody. After treatment of the slides with Elite ABC kit (Vector Laboratories), antigens were visualized with the 3,3'-diaminobenzidine tetrahydrochloride (DAKO) system. Counterstaining was performed with methyl green (DAKO).

**TABLE 2. Renal Function and Proteinuria After Ang II and NE Infusion**

	Creatinine Clearance, mL/min	Urinary Protein Excretion, mg/d
Control	3.2±0.2	9.7±1.3
Ang II	1.2±0.2*	28.1±7.2*
Ang II+hydralazine	1.3±0.2*	8.7±2.9
Ang II+losartan	3.1±0.2	10.1±2.2
NE	2.8±0.4	8.8±1.2
Ang II (0.25 mg · kg <sup>-1</sup> · d <sup>-1</sup> )	3.1±0.1	6.6±0.6

Rats were administered a continuous infusion of Ang II ( $0.7 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) or NE ( $0.25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) for 7 days and sacrificed. Some rats were administered losartan or hydralazine in the drinking water. The low-Ang II group was administered a subpressor dose of Ang II ( $0.25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) for 7 days. Note that NE did not significantly affect these values. Data from 5 to 8 plasma or urine samples are summarized in the table.

\* $P < 0.01$  vs sham-operated control.



**Figure 1.** Effect of continuous Ang II infusion on renal HO-1 expression. A and B, effect of Ang II infusion on HO-1 mRNA levels. A, representative Northern blot. B, summarized data from 4 to 6 Northern blots. C and D, effect of Ang II infusion on HO-1 protein levels. C, representative Western blot. In addition to a 32-kDa band, a 30-kDa band was occasionally detected. D, summarized data from 4 to 6 animals. \* $P < 0.05$  and \*\* $P < 0.01$  vs sham-operated control.

### Assay of HO Activity

Biliverdin reductase was crudely purified according to the method of Tenhunen et al.<sup>26</sup> The assay of HO activity was performed as described previously.<sup>23</sup>

### Statistical Analysis

Data are expressed as mean  $\pm$  SEM with ANOVA, followed by a multiple comparisons test for comparisons of initial data before expression as a percentage of the control. A value of  $P < 0.05$  was considered to be statistically significant.

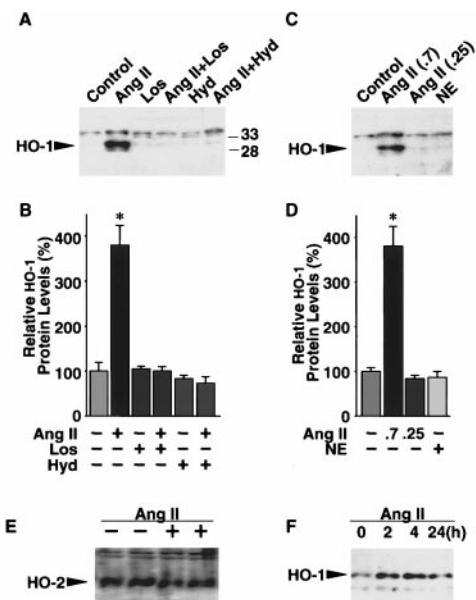
## Results

### Time Course of Systolic and Diastolic Blood Pressures and Heart Rate

The infusion of either Ang II and NE increased blood pressure and heart rate to similar extents (Table 1). Both hydralazine and losartan normalized Ang II-induced hypertension at day 7. The Ang II-induced increase in heart rate was completely normalized with losartan but only partially normalized with hydralazine (Table 1). Treatment with either hydralazine or losartan alone had no significant effect on blood pressure or heart rate (data not shown).

### Effect of Ang II and NE on Renal Function and Proteinuria

An infusion of Ang II at the dosage of  $0.7 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for 7 consecutive days resulted in a significant decrease in the glomerular filtration rate (GFR) determined through creatinine clearance (Table 2). Ang II also increased proteinuria to  $\approx 3$  times the control level. All of these laboratory values returned to normal levels after the administration of the specific AT<sub>1</sub> receptor blocker losartan. The nonspecific vasodilator hydralazine also normalized proteinuria but did not



**Figure 2.** Effects of antihypertensive agents, suppressor doses of Ang II, and NE on renal HO-1 protein expression. A and B, effects of losartan (Los) and hydralazine (Hyd). Both drugs completely blocked Ang II-induced HO-1 upregulation. C and D, effects of suppressor doses of Ang II ( $0.25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) and NE. Regardless of its hypertensive effect, NE infusion did not increase renal HO-1 expression, as did Ang II. Ang II increased HO-1 expression at  $0.7 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  but not at  $0.25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ . A and C, representative immunoblots. B and D, summarized data from 4 to 6 animals. \* $P < 0.01$  vs sham-operated control. E, representative Western blot of HO-2 protein from 3 independent experiments. F, effect of Ang II in vitro on renal tubular cells. IRPTCs were stimulated by  $100 \text{ nmol/L}$  Ang II for indicated time.

reverse the Ang II-induced decrease in GFR. In rats receiving either a suppressor dose of Ang II ( $0.25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) or NE, neither GFR nor the degree of proteinuria was different from that in normotensive rats.

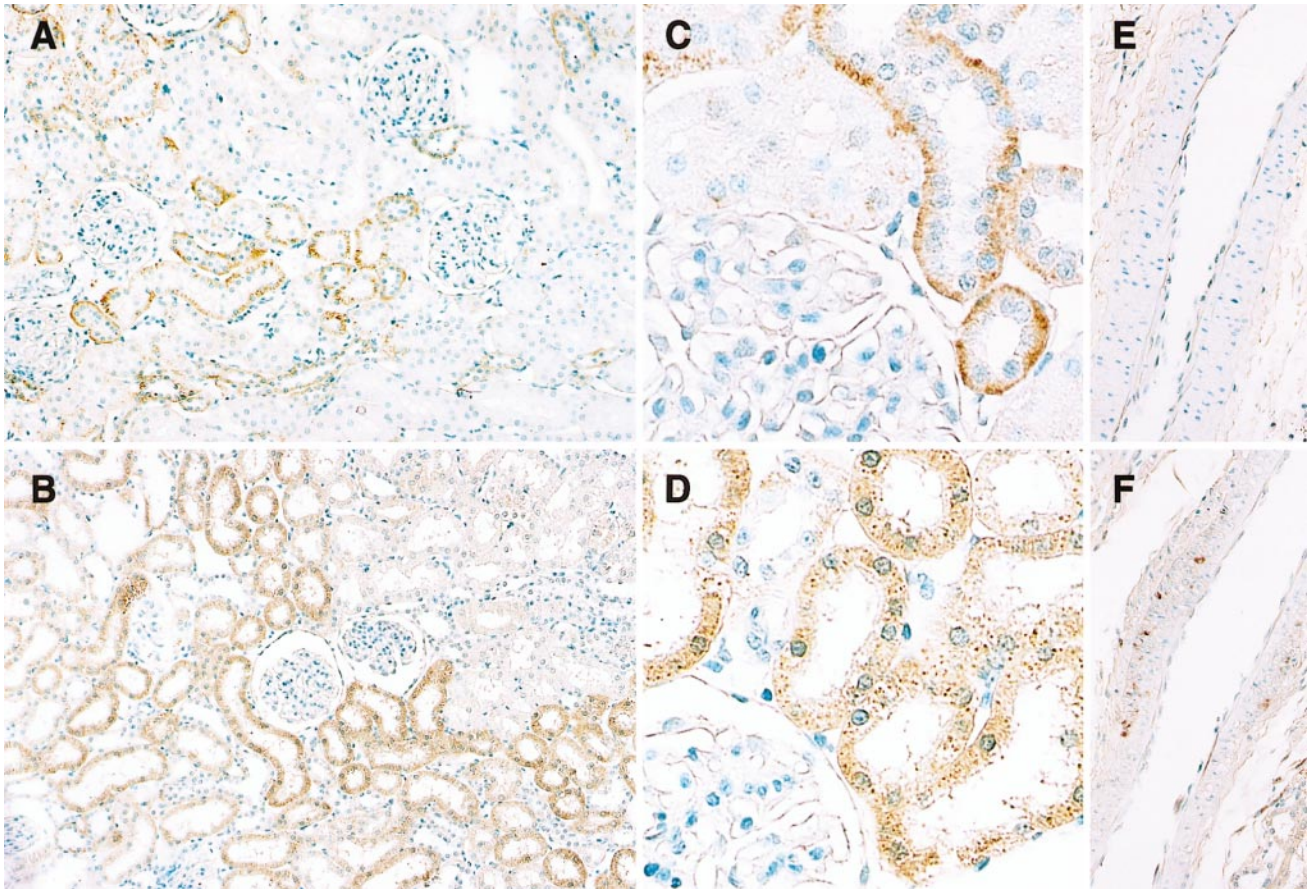
### Effect of Ang II Infusion on HO-1 Expression in the Kidney

We then investigated HO-1 regulation in the kidney of rats with Ang II-induced hypertension. HO-1 mRNA was significantly increased as early as 1 day after Ang II infusion and increased further for up to 7 days (Figures 1A and 1B). This increase in HO-1 mRNA expression was accompanied by an increase in HO-1 protein (Figures 1C and 1D). HO activity in the microsomal fraction was significantly elevated in the kidney of Ang II-infused rats compared with the control animals ( $5.4 \pm 0.8$  versus  $2.9 \pm 0.4 \text{ nmol bilirubin} \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$ ,  $n=4$ ,  $P < 0.05$ ).

### Effect of Vasodilators and NE on HO-1 Expression in the Kidney

Both hydralazine and losartan completely blocked the Ang II-induced HO-1 protein upregulation in the kidney (Figures 2A and 2B), which suggests that Ang II-induced HO-1 upregulation is a pressor-dependent event. This notion is supported by the fact that the suppressor dose of Ang II infusion did not increase HO-1 protein expression (Figures 2C and 2D). In contrast, however, NE infusion did not





**Figure 3.** Localization of HO-1. HO-1 staining in kidney of normotensive rats (A, C, and E) and Ang II-induced hypertensive rats (B, D, and F). A and B, HO-1 staining in glomerular and tubular regions (magnification  $\times 40$ ). C and D, higher-magnification microphotograph of HO-1 staining in tubular region. After Ang II infusion, HO-1 staining extended over a wider area to luminal side. E and F, microphotograph of HO-1 staining in renal artery at renal hilus (C to F magnification  $\times 100$ ).

upregulate HO-1 at all (Figures 2C and 2D). These findings suggest that the presence of both hypertension and high levels of circulating Ang II is necessary for the upregulation of renal HO-1 expression. Ang II infusion did not alter HO-2 expression in the kidney (Figure 2E). To examine whether Ang II has a direct effect on tubular HO-1 expression, IRPTCs were stimulated with 100 nmol/L Ang II. As shown in Figure 2F, Ang II stimulation significantly increased the HO-1 expression in IRPTCs (compared with control, 2 h  $337 \pm 58\%$  and 4 h  $340 \pm 25\%$ ;  $n=4$ ,  $P<0.05$ .)

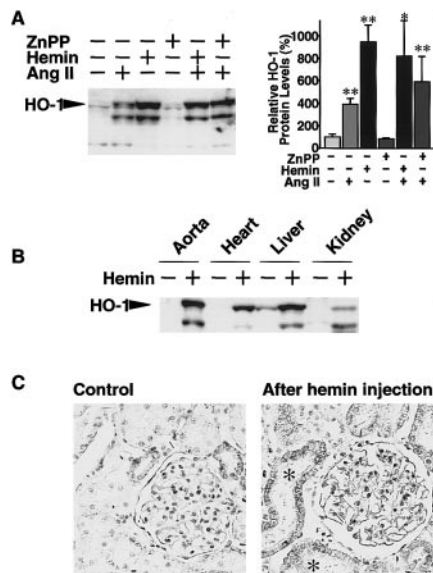
### HO-1 Immunohistochemistry

Immunohistochemistry of HO-1 revealed that HO-1 was present in both proximal and distal tubules in the kidney of normotensive rats. HO-1 staining in the tubular epithelial cells was more extensively distributed after Ang II infusion (Figures 3A and 3B). Under higher magnification, a distinct staining for HO-1 along the basal side of tubular epithelial cells was evident, whereas the luminal side of these cells was poorly stained in the kidney of normotensive rats (Figure 3C). In contrast, HO-1 staining was more extensive and was seen throughout the epithelial cells (Figure 3D) in the kidney of Ang II-infused rats. HO-1 expression was also increased in the arterial wall at the renal hilus in response to Ang II infusion (Figures 3E and 3F).

### Effects of Inducer and Inhibitor of HO on HO-1 Expression, Hemodynamics, Renal Function, and Proteinuria

To investigate the possible physiological relevance of Ang II-induced HO-1 upregulation in the kidney, either hemin or ZnPP was intraperitoneally administered to rats. As expected, hemin injection, but not ZnPP injection, upregulated renal HO-1 protein expression in the kidney (Figure 4A). HO-1 was also markedly upregulated by hemin injection in the other tested organs (Figure 4B). Immunohistochemistry revealed that hemin-induced HO-1 upregulation in the kidney was mainly seen in the tubular region (Figure 4C).

Hemin injection slightly decreased blood pressure in control rats, although it was not statistically significant. In addition, hemin normalized the Ang II-induced blood pressure elevation (Table 3). In contrast, ZnPP did not affect blood pressure in either control rats or those receiving Ang II (Table 3). Hemin or ZnPP injection did not significantly change GFR or proteinuria in normotensive rats. However, hemin increased GFR and decreased proteinuria in Ang II-infused rats. On the contrary, ZnPP augmented the Ang II-induced decrease in GFR and increase in proteinuria (Table 3).



**Figure 4.** Effect of HO-1 inducer hemin and HO-1 inhibitor ZnPP on renal HO-1 protein expression. A, effects of hemin and ZnPP on renal HO-1 expression. Left, representative immunoblots. Right, summarized data from 3 or 4 independent experiments ( $*P < 0.05$  and  $**P < 0.01$  compared with control, respectively). B, effects of hemin on HO-1 expression in other organs. Representative immunoblot of 2 independent experiments. C, immunohistochemistry of HO-1 in kidney of normotensive rats (left) and hemin-injected rats (right). Increased HO-1 staining was observed in tubular area in Ang II-infused rat kidney (\*).

## Discussion

In the present study, we showed that the continuous infusion of a pressor dose of Ang II, but not NE, resulted in a decrease in GFR and an increase in proteinuria. After an infusion of Ang II, renal HO-1 mRNA, protein, and activity were significantly increased. Immunohistochemistry demonstrated that HO-1 was mainly present in the basal side of tubular epithelial cells in the kidney of normotensive rats but that HO-1 was more extensively expressed in these areas in the

Ang II-infused rat. The administration of the HO inducer hemin ameliorated and the HO inhibitor ZnPP exacerbated renal injury induced by Ang II infusion, suggesting that HO-1 upregulation in the kidney of Ang II-infused rat may protect against Ang II-induced renal injury.

Our first observation was that Ang II infusion resulted in an increase in proteinuria and a decrease in GFR. The former was blocked by either losartan or hydralazine, but the latter was blocked only by losartan. Because NE infusion affected neither GFR nor proteinuria, it appears that in the Ang II-infused rat,  $AT_1$  receptor-mediated action of Ang II is responsible for the decreased GFR, whereas the synergistic action of Ang II and pressor overload is critical for the increased proteinuria.

We then found that HO-1 was upregulated in the kidney of rats rendered hypertensive with Ang II infusion but not in rats receiving the NE infusion. In addition, Ang II-induced HO-1 upregulation was blocked by either losartan or hydralazine. These data suggest that the synergistic action of circulating high levels of Ang II and pressor overload is critical for HO-1 upregulation. This pattern of HO-1 regulation in the kidney differs from that in the aorta, where HO-1 has been reported to be upregulated in response to hypertension per se.<sup>22</sup> The precise mechanisms of HO-1 upregulation remain unknown. Other investigators have reported elevated intrarenal Ang II levels in the Ang II-infused rat.<sup>27</sup> Because  $AT_1$  receptor is abundantly expressed in the proximal and distal tubules,<sup>28</sup> it is possible that HO-1 upregulation was due to the direct effect of intraluminal Ang II. In the present study, the finding that HO-1 was upregulated by Ang II in vitro in IRPTCs, in which  $AT_1$  receptor is expressed,<sup>24</sup> may support this hypothesis. It was of note that Ang II-induced HO-1 upregulation occurred in parallel with increased proteinuria. It has been reported that tubular protein overload upregulates proinflammatory mediators in the renal tubules.<sup>29,30</sup> Therefore, it is possible that HO-1 was upregulated in response to increased proteinuria as an anti-inflammatory defense.

Occasionally, when HO-1 was upregulated, a 30-kDa immunoreactive band appeared on immunoblot analysis (Figures 1C, 2A, 2C, 4A, and 4B). Because that 30-kDa band emerged when HO-1 protein was markedly increased in IRPTCs with the infection of an adenoviral vector containing the rat HO-1 gene (N. Ishizaka, unpublished data, 1999), this band most likely is HO-1 protein that has undergone post-translational modification or degradation.

The possible physiological importance of renal HO-1 upregulation in the Ang II-infused rat is 3-fold. First, like nitric oxide synthase,<sup>31</sup> HO-1 may partially counteract the vasoconstrictor influence of elevated circulating Ang II via the CO-mediated activation of soluble guanylate cyclase. Second, HO may activate 70-pS potassium channel in the rat thick ascending limb<sup>32</sup> via CO production. Potassium recycling via the activation of potassium channel plays an important role in the provision of an adequate supply of potassium to the  $Na^+, K^+, Cl^-$  cotransporter. Therefore, Ang II may play a role in sodium reabsorption through the activation of potassium channel as well as the activation of

**TABLE 3. Effect of Hemin and ZnPP on Blood Pressure and Renal Function in Ang II-Infused Rats**

	Systolic Blood Pressure, mm Hg	Creatinine Clearance, mL/min	Urinary Protein Excretion, mg/d
Control	135±6	3.2±0.2	9.7±1.3
Ang II	201±4†	1.2±0.2†	28.1±7.2†
Hemin	136±3	2.9±0.3	6.6±1.5
ZnPP	140±6	3.1±0.2	7.1±1.4
Ang II+hemin	136±8‡	2.4±0.2*‡	9.3±4.5‡
Ang II+ZnPP	200±12†	0.8±0.1†‡	52.5±13.0†‡

Blood pressure data from 6 to 8 rats and data from 6 to 10 plasma or urine samples are summarized in the table. Neither hemin nor ZnPP had any effect on systolic blood pressure. In contrast, hemin plus Ang II brought blood pressure down to control levels, whereas ZnPP did not. Hemin plus Ang II decreased the Ang II-associated decrease in creatinine clearance and increase in urinary protein excretion.

\* $P < 0.05$  and † $P < 0.01$  compared with the control.

‡ $P < 0.05$  compared with Ang II-infused rats.



the Na<sup>+</sup>,K<sup>+</sup>,Cl<sup>-</sup> cotransporter.<sup>33</sup> Third, the induction of HO-1 may be an adaptive response that protects the kidney against renal insults. HO-1 induction exerts a renoprotective effect in animal models of rhabdomyolysis<sup>19</sup> and nephrotoxic nephritis.<sup>21</sup> In the present study, the administration of ZnPP to Ang II-infused rats further decreased GFR and increased proteinuria. In contrast, the administration of hemin to Ang II-infused rats ameliorated GFR decrease and lessened proteinuria. These findings support the hypothesis that Ang II-induced HO-1 upregulation in the kidney provides renoprotection. Because hemin administration completely suppressed Ang II-induced blood pressure elevation, its antiproteinuric effect may also be attributed to its antihypertensive effect, as in the case with hydralazine. This antihypertensive effect of hemin appears to be consistent with previous reports showing that hemin or HO substrates normalized blood pressure in other types of hypertension.<sup>34,35</sup> The mechanisms of the antihypertensive effect of HO-1 upregulation may involve CO-mediated activation of soluble guanylate cyclase<sup>36</sup> and the activation of calcium-activated potassium channels.<sup>37</sup>

In summary, the continuous infusion of Ang II induced a renal injury in both a pressor-independent (decreased GFR) and a pressor-dependent (proteinuria) manner. HO-1 was upregulated in the kidney of rats with Ang II-induced hypertension. The findings that HO-1 induction ameliorated and HO inhibition augmented Ang II-induced renal injury suggest that Ang II-induced upregulation of HO-1 may act renoprotectively against renal injury evoked by chronic Ang II infusion.

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