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Angiotensin II induces renal oxidant stress in vivo and heme oxygenase-1 in vivo and in vitro[1]

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Angiotensin II induces renal oxidant stress in vivo and heme oxygenase-1 in vivo and in vitro.

Background. Angiotensin II is strongly incriminated in progressive renal injury. There is recent evidence that angiotensin II induces oxidative stress in vitro. We examined the capacity of angiotensin II to induce oxidative stress in vivo and the functional significance of such stress. The capacity of angiotensin II to induce the oxidant-sensitive gene heme oxygenase (HO) in vivo and in vitro was also examined.

Methods. Angiotensin II was administered via mini-osmotic pumps to rats maintained on standard diets. Indices of oxidative stress, including thiobarbituric acid reactive substance, carbonyl protein content, and HO activity, were determined. Indices of oxidative stress and functional markers were also determined in the DOCA salt model. The effect of angiotensin II was studied in rats maintained on antioxidant-deficient diets so as to examine the functional significance of oxidative stress induced by angiotensin II. We also explored the inductive effect of angiotensin II on HO in vivo and whether such actions occur in vitro.

Results. Angiotensin II administered in vivo increased kidney content of thiobarbituric acid reactive substances protein carbonyl content, and HO activity. These indices were not present in the kidney of rats treated with DOCA salt for three weeks. Such oxidative stress was functionally significant, since the administration of angiotensin II to rats maintained on a prooxidant diet demonstrated increased proteinuria and decreased creatinine clearance. The stimulatory effect on HO activity was due to induction of HO-1 mRNA, with HO-2 mRNA remaining unchanged. Expression of HO-1 was localized to the renal proximal tubules in vivo. We also demonstrate that angiotensin II at concentrations of 10^{-8} and 10^{-7} mol/L induces expression of HO-1 mRNA in LLC-PK1 cells.

Conclusions. Angiotensin II induces oxidative stress in vivo, which contributes to renal injury. This study also demonstrates that angiotensin II induces renal HO activity caused by upregulation of HO-1 in renal proximal tubules. Finally, angiotensin II directly induces HO-1 in renal proximal tubular epithelial cells in vitro.

[1]**See Editorial by Meyer, p. 458**

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Angiotensin II is incriminated in the pathogenesis of renal and cardiovascular injury [1–9]. Angiotensin II-induced renal injury may arise from its hemodynamic or nonhemodynamic actions, or from such effects of angiotensin II acting in concert [1–6]. Hemodynamic actions include elevation in systemic arterial pressure, alterations in intraglomerular hemodynamics, and alterations in interstitial hemodynamics; nonhemodynamic actions of angiotensin II include increased expression of assorted growth factors and protooncogenes, aberrant growth responses, and elaboration of inflammatory and fibrogenic cytokines [1–7].

A nonhemodynamic action of angiotensin II, recently described in smooth muscle cells and relevant to renal injury, is the induction of oxidative stress [8, 9]. Such oxidative stress in smooth muscle cells may arise from the stimulation of the membrane-bound NADH/NADPH oxidase [8, 9], an enzyme also present in endothelial cells [10], and which leads to increased generation of superoxide anion [8, 9]. Such angiotensin II-induced oxidative stress is incriminated in altered vascular responses that contribute to systemic hypertension and vascular injury [8–13]. Thus, the stimulation of reactive oxygen species represents an action of angiotensin that, while "nonhemodynamic" in terms of its biochemical origin, interfaces with and relates to the hemodynamic effects of angiotensin II.

More recently, a similar oxidative effect of angiotensin II has been described in mesangial cells and LLC-PK1 cells in vitro [14, 15]. Besides being present in endothelial and smooth muscle cells, a membrane-bound NADPH/NADH oxidase is also present in cells intrinsic to the kidney such as mesangial cells [16], glomerular podocytes [17], and proximal tubular epithelial cells [18]. In response to angiotensin II, mesangial and LLC-PK1 cells demonstrate increased production of superoxide anion emanating from induced NADPH/NADH activity [14, 15]; such biochemical responses influence cellular proliferation in vitro and are inferentially linked to the role of angiotensin II in progressive renal disease [14, 15].

In the present study, we questioned whether angioten-

sin II induces oxidative stress in vivo in the kidney and whether such oxidant stress may be incriminated in renal injury induced by angiotensin II in vivo. In the course of these studies and as an index of oxidative stress, we examined the effect of angiotensin II on heme oxygenase (HO) activity. HO exists in at least two isoforms, HO-1 and HO-2 [19, 20]. HO-1 is widely induced by oxidative stress, cytokines, heavy metals, advanced-glycated end products, while the constitutive isoform, HO-2, is induced by a quite limited number of stimuli that includes glucocorticoids [19, 20]. We thus examined the basis for the induction of HO activity and localized the site within the kidney where such induction occurred.

METHODS

Chronic administration of angiotensin II by mini-osmotic pump

Angiotensin II (Sigma, St. Louis, MO, USA) was administered to male Sprague-Dawley rats subcutaneously via mini-osmotic pumps (Alzet Corp., Palo Alto, CA, USA) at two different doses [2]. Rats were maintained on standard rat chow (Ralston Purina Co., St. Louis, MO, USA) and tap water ad libitum. The higher dose protocol administered angiotensin II at 200 ng/min/300 g body weight for up to two weeks, while the lower dose protocol used angiotensin II at 60 ng/min/300 g body weight for four weeks. Angiotensin II was dissolved in lactated Ringer's solution and loaded into two- or four-week mini-osmotic pumps according to the recommendations of the manufacturer. These pumps, containing angiotensin II or vehicle, were placed into subcutaneous tissue via a midscapular incision in rats anesthetized with Methohexitol (50 mg/kg body weight), the latter administered intraperitoneally. Rats were subsequently housed individually for one week to prevent pump loss. In these and other studies, systolic blood pressures were measured in awake, restrained rats via tail cuff plethysmography [21]. Twenty-four-hour metabolic collections were obtained in a standard fashion, and urinary concentration of protein was determined by the Coomassie method [21]. Concentrations of creatinine in serum and urine were analyzed using a Beckman II Creatinine Analyzer [21].

Model of systemic hypertension induced by DOCA salt

Uninephrectomy was performed in male Sprague Dawley rats anesthetized with intraperitoneal methohexital (50 mg/kg body weight). After uninephrectomy, rats were allowed to drink normal saline and were injected weekly with deoxycorticosterone acetate (DOCA) in olive oil (10 mg/rat) administered subcutaneously [22]; sham-nephrectomized rats received tap water. After three weeks, rats were sacrificed for the determination of indices of renal oxidative stress. In additional cohorts of DOCA salt-treated and control rats, indices of renal function and systolic blood pressure were determined after three weeks.

Chronic administration of angiotensin II in rats maintained on diets deficient in antioxidants

To examine the functional effects of angiotensin II-induced oxidative stress, the effects of angiotensin II were studied in rats maintained on a diet deficient in vitamin E and selenium [21, 23]; this dietary deficiency uncovers the functional effects of oxidative phenomena since deficiency of selenium impairs glutathione peroxidase-dependent scavenging of H_2O_2, while vitamin E deficiency removes the endogenous antioxidant in lipid bilayers in plasma and intracellular membranes. Weanling rats were placed on vitamin E and selenium-deficient and control diets, and mini-osmotic pump placement was performed two weeks following the initiation of this diet. Angiotensin II was delivered at a rate of 200 ng/min/300 g body weight for two weeks. In additional studies, weanling rats were maintained on the control and deficient diets for two weeks and were then subjected to administration of vehicle infused at an identical rate via mini-osmotic pumps for two weeks.

Indices of oxidative stress

Three indices of oxidative stress were employed: lipid peroxidation, protein carbonyl content, and activity of the oxidant-inducible enzyme HO. Lipid peroxidation was determined by measurement of thiobarbituric acid reactive substances (TBARS), as described in our previous studies [23]. HO activity was determined in kidney microsomes in which the rate of formation of bilirubin was assayed, as previously described [24]. Protein carbonyl content was determined by the method based on 2,4-dinitrophenylhydrazine (DNPH) [25]. A soluble protein fraction of the kidney was separated into aliquots that were incubated for one hour with either 2 mol/L HCl or 10 mmol/L 2,4-DNPH dissolved in 2 mol/L HCl. Following precipitation with trichloroacetic acid, the pellet was washed and then dissolved in guanidine hydrochloride. The difference in absorbance between samples treated with 2,4-DNPH in 2 mol/L HCl, and the sample treated with 2 mol/L HCl alone was determined at 366 nmol. Data are expressed as nanomoles of carbonyl groups per milligram of protein.

Expression of HO-1 and HO-2 by Northern analysis

Total RNA from rat kidney and from cultured cells was isolated using a modification of the guanidinium-isothiocyanate/cesium chloride method, and Northern blot analysis was performed, as previously described [23, 24]. Aliquots (20 μg) of total RNA were separated by electrophoresis. Autoradiograms were quantitated by computer-assisted videodensitometry, and results were standardized by the method of Correa-Rotter, Mariash,

and Rosenberg [26]. This established method of standardization corrects for any variability due to loading and transfer, and factors the optical density of the message for the given gene with the optical density of the 18S rRNA, the latter obtained on a negative of the ethidium bromide-stained nylon membrane. Northern analyses were undertaken using cDNA probes for mouse HO-1 and human HO-2 [23, 24].

Expression of HO-1 by immunohistochemical analysis

Immunohistochemical analysis was performed as previously described on tissue sections fixed in 10% neutral buffered formalin and embedded in paraffin [27]; expression of HO-1 was localized using a monoclonal antibody (OSA-111; Stressgen, Victoria, BC, Canada) as the primary antibody, a polyclonal goat anti-mouse IgG as the secondary antibody (SAB-100; Stressgen), and diaminobenzidine as substrate for localization.

Expression of HO-1 in LLC-PK1 cells exposed to angiotensin II

LLC-PK1 cells were maintained in culture in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum, as previously described [28]. Cells were seeded onto 15 cm plates and grown to approximately 60% confluency. Media were replaced by serum-free DMEM for 24 hours, after which cells were incubated in either serum-free DMEM alone or DMEM containing 10^{-8} or 10^{-7} mol/L angiotensin II for 48 hours. After the first 24 hours, the media were replaced with freshly prepared media containing angiotensin II or the control medium. RNA was subsequently extracted from these cells.

Statistics

Data are expressed as means ± SEM. For comparison of two groups, the unpaired Student *t*-test or the Mann–Whitney test was used as appropriate. Values are considered statistically significant for $P < 0.05$.

RESULTS

Effect of angiotensin II on renal function and renal oxidative stress

Administration of the higher dose of angiotensin II significantly increased systolic blood pressure and urinary protein excretory rates and significantly decreased creatinine clearance (Table 1). Such doses of angiotensin II imposed oxidative stress on the kidney in vivo in that all markers of oxidative injury—TBARS, protein carbonyl content, and the activity of the oxidant-sensitive enzyme, HO—were significantly increased (Fig. 1).

Administration of the lower dose of angiotensin II significantly increased urinary protein excretion and systolic blood pressure while tending to decrease creatinine clearance (Table 2). Such doses of angiotensin II also imposed oxidative stress on the kidney in vivo, as indicated by significant elevations in TBARS and HO activity; however, protein carbonyl content was not increased (Fig. 2).

Table 1. Functional effects of angiotensin II administered by mini-osmotic pump (200 ng/min/300 g body wt)

	Vehicle ($N = 12$)	Angiotensin II ($N = 10$)
Body weight *g*	271 ± 5	208 ± 12[a]
C_{Cr} *mL/min*	3.04 ± 0.25	1.52 ± 0.14[a]
C_{Cr}/100 g body weight	1.13 ± 0.10	0.75 ± 0.08[a]
Proteinuria *mg/24 hr*	22 ± 2	105 ± 12[a]
Blood pressure *mm Hg*	134 ± 4	220 ± 10[a]

Data are means ± SEM. C_{Cr} is creatinine clearance.
[a] $P < 0.05$ vs. vehicle

Renal oxidative stress in the DOCA salt model of hypertension and renal injury

To determine whether oxidative stress occurs in the kidney in another model of systemic hypertension and renal injury, we measured these three indices in the kidney in a model of hypertension that is considered angiotensin II-independent, namely, the DOCA salt model. Systolic blood pressure and indices of renal function and other alterations in the DOCA salt model are summarized in Table 3. As shown, this model demonstrated marked elevation in systolic blood pressure, markedly increased rates of urinary protein excretion, and a reduction in creatinine clearance. Statistical analyses by analysis of variance of these parameters in the DOCA salt model (Table 3) and in rats subjected to the higher dose of angiotensin II (Table 1) demonstrated that systolic blood pressures, rates of urinary protein excretion, and creatinine clearances (absolute or factored for body weight) were not significantly different in angiotensin II-infused and DOCA salt-treated rats. In additional cohorts of DOCA salt-treated and control rats, indices of oxidative stress, including HO activity, were measured after three weeks. As depicted in Figure 3, none of these indices were elevated. Thus, at this time point in this model, oxidant stress does not occur despite markedly elevated systolic blood pressure and proteinuria and decreased creatinine clearance.

Functional effect of chronic angiotensin II infusion in rats maintained on an antioxidant-deficient diet

To determine the functional effect of increased oxidative stress imposed by angiotensin II in vivo, the effect of angiotensin II was examined in rats maintained on a diet deficient in antioxidants. The baseline body weight, creatinine clearance, urinary protein excretory rates, and systolic blood pressure in control rats and rats maintained on the deficient diets were not significantly differ-

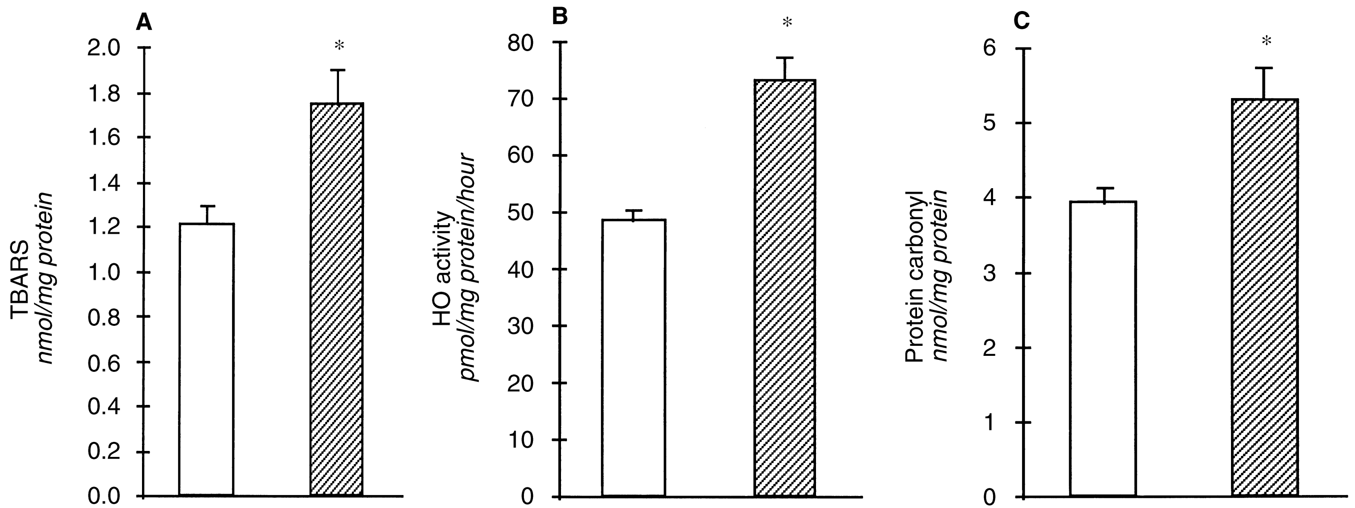

Fig. 1. Effect of angiotensin II (▨; 200 ng/min/300 g body weight; $N = 8$) or vehicle (□; $N = 7$) infused by mini-osmotic pump on indices of oxidative stress (TBARS, HO activity, and protein carbonyl content) in the kidney (*$P < 0.05$).

Table 2. Functional effects of angiotensin II administered by mini-osmotic pump (60 ng/min/300 g body weight)

	Vehicle ($N = 6$)	Angiotensin II ($N = 8$)
Body weight *g*	346 ± 9	314 ± 5[a]
C_{Cr} *mL/min*	2.97 ± 0.25	2.48 ± 0.08
C_{Cr}/100 g body weight	0.87 ± 0.08	0.79 ± 0.03
Proteinuria *mg/24 hr*	43 ± 9	107 ± 26[a]
Blood pressure *mm Hg*	134 ± 3	212 ± 7[a]

Data are means ± SEM. C_{Cr} is creatinine clearance.
[a]$P < 0.05$ vs. vehicle

Table 3. Functional studies in control and DOCA salt-treated rats

	Control ($N = 6$)	DOCA salt ($N = 7$)
Body weight *g*	347 ± 6	321 ± 12
C_{Cr} *mL/min*	3.00 ± 0.17	1.99 ± 0.15[a]
C_{Cr}/100 g body weight	0.87 ± 0.05	0.65 ± 0.04[a]
Proteinuria *mg*/24 hr	23 ± 2	341 ± 116[a]
Blood pressure *mm Hg*	133 ± 2	194 ± 6[a]

Data are means ± SEM. C_{Cr} is creatinine clearance.
[a]$P < 0.05$ vs. vehicle

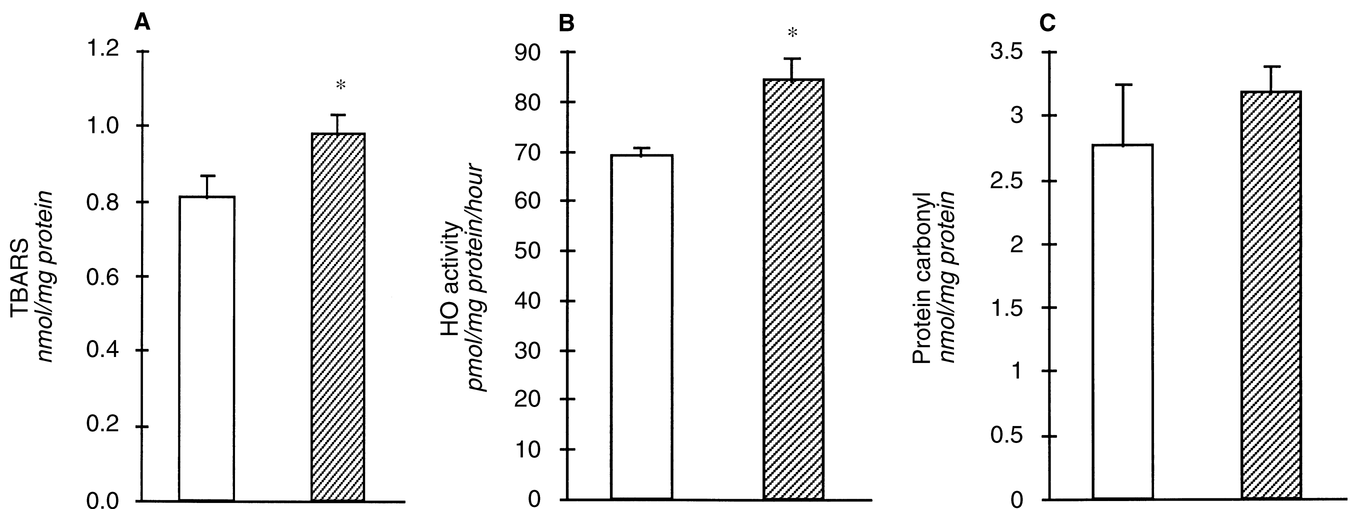

Fig. 2. Effect of angiotensin II (▨; 60 ng/min/300 g body weight; $N = 8$) and vehicle (□; $N = 6$) infused by mini-osmotic pump on indices of oxidative stress (TBARS, HO activity, and protein carbonyl content) in the kidney (*$P < 0.05$).

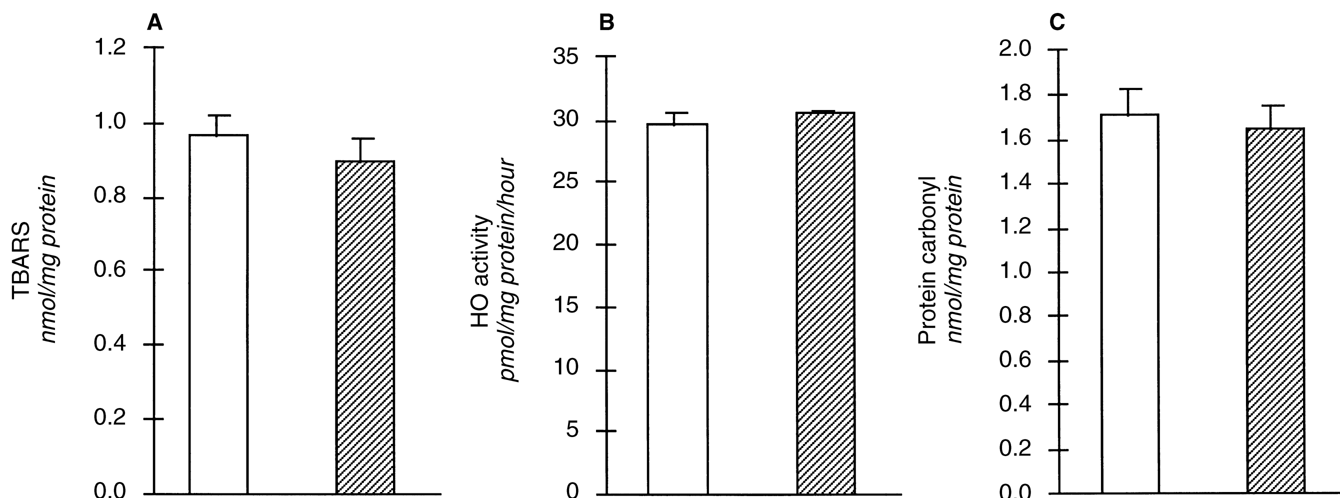

Fig. 3. Indices of oxidative stress (TBARS (*A*), HO activity (*B*), and protein carbonyl (*C*) content) in the kidney in the DOCA salt model. Symbols are: (□) Control, $N = 5$; (▨) DOCA salt, $N = 8$. P = NS.

Table 4. Functional parameters in rats maintained on control and deficient diets infused with angiotensin II by mini-osmotic pump

	Control diet ($N = 8$)	Deficient diet ($N = 10$)
Body weight *g*	159 ± 5	136 ± 5[a]
C_{Cr} *mL/min*	1.20 ± 0.06	0.79 ± 0.07[a]
C_{Cr}/100 g body weight	0.75 ± 0.04	0.58 ± 0.06[a]
Proteinuria *mg/24 hr*	4.8 ± 1.9	20.6 ± 5.1[a]

Data are means ± SEM. C_{Cr} is creatinine clearance.
[a]$P < 0.05$ vs. control diet

Table 5. Functional parameters in rats maintained on control and deficient diets infused with vehicle by mini-osmotic pump

	Control diet ($N = 6$)	Deficient diet ($N = 6$)
Body weight *g*	187 ± 5	170 ± 5[a]
C_{Cr} *mL/min*	1.9 ± 0.2	1.7 ± 0.3
C_{Cr}/100 g body weight	1.0 ± 0.1	1.0 ± 0.2
Proteinuria *mg/24 hr*	1.9 ± 0.2	1.3 ± 0.2

Data are means ± SEM. C_{Cr} is creatinine clearance.
[a]$P < 0.05$ vs. control diet

ent (data not shown). The infusion of angiotensin II diminished creatinine clearances (expressed in absolute terms or factored for body weight) and increased urinary protein excretory rates in rats maintained on the deficient diets (Table 4). These effects were due to angiotensin II imposed in the setting of this dietary manipulation since rats maintained on the control and deficient diets demonstrated comparable creatinine clearances and urinary protein excretory rates when vehicle was infused over a similar time period (Table 5).

Systolic blood pressure was measured on days 4, 8, 11, and 14 after the administration of angiotensin II in rats maintained on the control and deficient diets. There were no differences between the groups except on the final measurement (day 14) when systolic blood pressures in rats on the deficient diet were marginally higher, albeit significantly so, as compared with such pressures in rats on the control diet and infused with angiotensin II (188 ± 6 vs. 217 ± 9 mm Hg, $P < 0.05$).

Induction of HO-1 in the kidney in vivo by angiotensin II

We further examined the induction of HO by angiotensin II in the kidney, specifically, questioning whether induction of HO-1 contributed to such increments. HO consists of at least two isoforms: the oxidant-inducible one, HO-1, and the constitutively expressed isoform, HO-2. We thus determined which of these two isoforms were induced. Northern analyses demonstrated a striking induction in HO-1 in the kidney following the infusion of angiotensin II: HO-1 mRNA was increased sevenfold (1.4 ± 0.2 vs. 9.7 ± 1.6, standardized OD units, $P < 0.05$; Fig. 4). In these studies, no increment in HO-2 mRNA was observed in the kidney (5.0 ± 0.8 vs. 5.0 ± 0.5, OD units, P = NS; data not shown).

The site of induction of HO-1 was localized by immunohistochemical analyses (Fig. 5). Vehicle-infused rats demonstrated no induction of HO-1 in the kidney (Fig. 5A), whereas the kidneys in rats infused with angiotensin II demonstrated marked induction of HO-1, which localized largely to renal proximal tubules (Fig. 5B).

Induction of HO-1 in renal epithelial cells in vitro by angiotensin II

To determine whether angiotensin II exerts a direct inductive effect on cellular expression of HO-1 mRNA the gene expression was evaluated in LLC-PK1 cells exposed to angiotensin II in vitro during conditions that

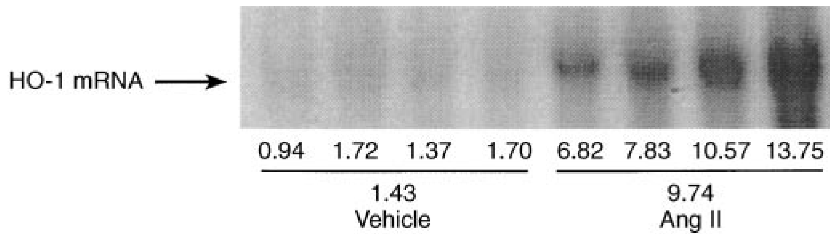

Fig. 4. Heme oxygenase-1 (HO-1) mRNA expression in the kidney in rats subjected to vehicle or angiotensin II (Ang II; 200 ng/min/300 g body weight) infused by mini-osmotic pump. Each lane represents mRNA extracted from a single kidney from an individual rat. The individual and mean standardized densitometric readings are provided below the Northern analysis.

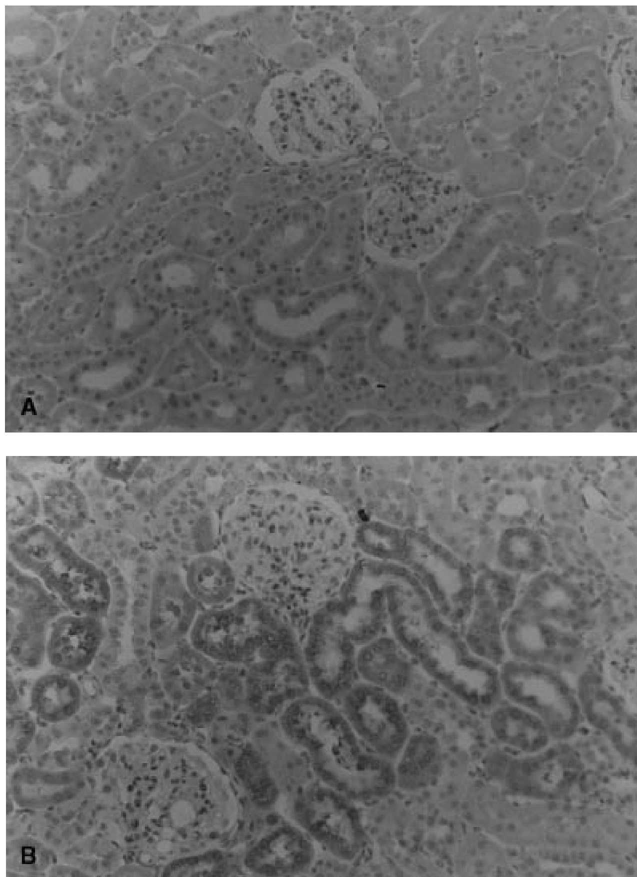

Fig. 5. Localization of HO-1 expression by immunoperoxidase in the kidney in rats subjected to vehicle (*A*) or angiotensin II (200 ng/min/300 g body weight; *B*) infused by mini-osmotic pump. Original magnification ×200.

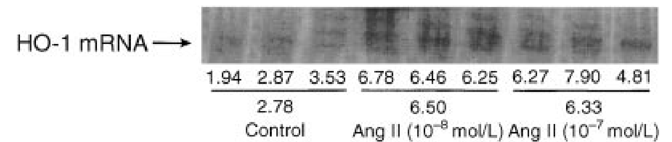

Fig. 6. HO-1 mRNA in LLC-PK1 cells exposed to angiotensin II at increasing concentrations. Each lane represents mRNA extracted from an individual plate under the stated conditions: Control, angiotensin II (Ang II) at concentrations of 10^{-8} and 10^{-7} mol/L. The individual and mean standardized densitometric readings are provided below the Northern analysis.

varied the concentrations of angiotensin II and the duration of exposure. Exposure of LLC-PK1 cells to angiotensin II at concentrations of 10^{-8} and 10^{-7} mol/L for 48 hours induced a 2.3-fold in HO-1 (Fig. 6).

DISCUSSION

Using diverse markers of oxidative injury—an index of oxidation of lipid (TBARS), an index of protein oxidation (carbonyl content), and the induction of a redox-sensitive enzyme (HO)—we provide evidence that the administration of angiotensin II in vivo induces oxidative stress in the kidney. These data complement the recent demonstration that the exposure of mesangial cells [14] or renal epithelial cells such as LLC-PK1 [15] to angiotensin II induces oxidative stress in vitro because of induction of NADH/NADPH oxidase.

Angiotensin II exerts effects on the kidney that are ascribable to hemodynamic and nonhemodynamic actions, and both actions contribute to the involvement of angiotensin II in progressive renal injury [1–6]. In an attempt to determine the specificity of angiotensin II per se or the effects induced by angiotensin II in vivo (systemic hypertension, proteinuria, and reduced creatinine clearance) in provoking oxidant stress, we examined indices of oxidative stress in the DOCA salt model of systemic hypertension, one in which such functional changes occur in a setting in which angiotensin II is systemically suppressed. As shown in Table 3, the DOCA salt model, when studied at three weeks, exhibits elevations in systolic blood pressure, reductions in creatinine clearance, and elevations in urinary protein excretion, which are all comparable to that observed in the angiotensin II-infused rats. Thus, indices of oxidative stress in the DOCA salt model studied after three weeks remained unaltered in the face of marked systemic hypertension, proteinuria, and diminished GFR. Nevertheless, it must be pointed out that these findings do not negate a role for systemic hypertension or proteinuria in inducing oxidative stress and HO-1, since it is possible that exposure to DOCA salt for a period longer than three weeks may be required to evince such effects. It is conceivable that at time points later than three weeks, when renal injury is more established, indices of oxidative stress and induction of HO may occur in the DOCA salt model. It is

also possible that these alterations in blood pressure and proteinuria may exert oxidative stress and expression of HO-1, but such effects may require the presence of certain "threshold" amounts of angiotensin II. The latter may not be present in the DOCA salt model since it is associated with systemic suppression of angiotensin II.

To examine the functional significance of oxidative stress induced by angiotensin II, the effects of angiotensin II were studied in a dietary deficiency state in which the kidney is rendered prooxidant. The application of a diet deficient in vitamin E and selenium promotes a prooxidative state through the following mechanisms [21, 23]. Selenium is a cofactor for glutathione peroxidase, one of the two hydrogen peroxide-degrading enzymes (the other being catalase), and vitamin E is the major antioxidant resident in lipid bilayers. Thus, a combined deficiency of these two dietary constituents fosters the accumulation of hydrogen peroxide and its attendant effects on lipid bilayers as well as other cellular effects. This dietary manipulation was employed at a sufficiently early stage such that the recognized adverse effects of this dietary deficiency per se on creatinine clearance and proteinuria were not evinced. Chronic infusion of angiotensin II lowered creatinine clearance and increased urinary protein excretion in rats maintained on the deficient diet as compared with rats on the control diets. These parameters were not different in rats on these diets prior to the initiation of angiotensin II, nor were these parameters different in vehicle-infused rats maintained on the control and deficient diets. Thus, the oxidative effect of angiotensin II appears functionally significant since indices of injury—creatinine clearance and proteinuria—are exacerbated by angiotensin II in kidneys rendered prooxidant.

Rates of urinary protein excretion in response to chronic administration of angiotensin II were lower in rats maintained on the control diets (antioxidant sufficient diet) in the antioxidant-deficiency study (Table 4) as compared with rats on standard Purina rat chow (Table 1). The compositions of these diets differ markedly in many constituents. For example, the control diet for the antioxidant deficiency study contains lower amounts of protein than Purina rat chow and is based on a different protein source, Torula yeast. These and other differences in dietary composition contribute to the lower rates of urinary protein excretion in response to angiotensin II. Additionally, rats used in the antioxidant-deficiency study were younger and lighter in weight, the latter consideration leading to a lesser absolute amount of angiotensin II being infused in the antioxidant-deficiency study. Such differences, in addition to those pertaining to the respective diets, underlie the differences in urinary protein excretion.

Angiotensin II may induce oxidant stress via other mechanisms besides activation of the NADPH/NADH oxidase system and attendant generation of superoxide anion. For example, angiotensin II markedly increases urinary excretion of proteins, and among the latter are transferrin and other transition metal-bearing proteins [29]; such transition metals as iron and copper are potent catalysts for oxidative stress [30]. Additionally, angiotensin II may stimulate the elaboration of a number of cytokines, many of which are prooxidant [6, 31, 32].

Generation of reactive oxygen species by angiotensin II may contribute to progressive renal injury through diverse mechanisms [31–33]. By their vasoactive effects on the afferent and efferent arterioles and the mesangium, oxidants may induce glomerular hemodynamic alterations, which contribute to progressive renal disease. Oxidants can compromise glomerular permselective properties, thereby leading to increased urinary excretion of proteins; this latter alteration may be damaging to the renal tubular epithelium [30]. Relevant to these considerations is our finding that proteinuria was greater, and creatinine clearance was lower, following the chronic administration of angiotensin II in rats on the deficient diet as compared with those on the control diet. Such findings suggest that oxidative stress arising from angiotensin II may influence renal hemodynamics and glomerular permselective properties. Oxidants can exert direct cytotoxicity to cells in the glomerular, vascular, and tubulointerstitial compartments. Additionally, there is mounting evidence that key steps in the elicitation of an inflammatory response may be up-regulated by oxidants [31–33]: Activation of endothelial and epithelial cells and the elaboration of proinflammatory and chemotactic substances may also be induced by oxidants. More specifically, adhesion molecules (intercellular adhesion molecule-1 and vascular cellular adhesion molecule-1), selectins (E-selectin, P-selectin), the T-cell chemoattractant (RANTES), the monocyte chemoattractant (MCP-1), and the fibrogenic cytokine (transforming growth factor-β1), may be up-regulated by oxidative stress [31–33]. Indeed, the transcription factor that is involved in the up-regulation of numerous proinflammatory species, namely, nuclear factor-κB (NF-κB), can be induced by oxidative stress [31, 33]. Interestingly, several of these participants in inflammation, in particular, NF-κB, are inducible by angiotensin II [6, 34–36]; and in models of immune complex nephritis and antiglomerular basement disease, pathogenetic roles have been recently assigned to angiotensin II [36, 37]. Thus, angiotensin II—physiology's archetypal, vasoactive substance—is now regarded as a promoter of inflammatory and immune renal injury. Such effects of angiotensin II may reflect, at least in part, the prooxidant actions of angiotensin II.

Heme oxygenase-1 was employed as a marker of oxidative stress in light of the recent evidence that the infusion of angiotensin II induces HO-1 in the vasculature [38]. Extrapolating from the recognized effects of HO

in other systems [19, 20], it is possible that such induction in the vasculature may confer antioxidant, anti-inflammatory, and vasodilatory properties. Our studies demonstrate marked increments in HO activity in the kidneys of rats subjected to angiotensin II infusion. Such increased HO activity may reflect either increments in HO-1, HO-2, or both. HO is the isozyme induced by a number of stimuli, many of which share the common effect of influencing cellular redox; HO-2 is the constitutively expressed isozyme [19, 20]. We demonstrate that the increase in HO activity is due to HO-1 since Northern analyses revealed induction of HO-1 mRNA without any significant change in HO-2 mRNA. Additional support for the induction of HO-1 as the basis for increased HO activity was provided by immunohistochemical studies in which the localization of HO-1 was undertaken. In these studies, induction of HO-1 was observed largely in the proximal tubules in animals infused with angiotensin II. The functional significance of the expression of HO-1 in the kidney in vivo in response to angiotensin II invites certain speculations. Such induction of HO-1 may represent an antioxidant response that mitigates the severity of oxidative stress and attendant injury induced by angiotensin II [19, 20]. It is also possible that the expression of HO-1 in response to the administration of angiotensin II in vivo represents an anti-inflammatory response that limits the extent of inflammation induced by angiotensin II [19, 20, 39].

To determine whether angiotensin II would directly elicit such effects in renal epithelial cells, we studied proximal tubular epithelial cells in culture exposed to angiotensin II. Under certain conditions, we observed more than a twofold induction of HO-1 in response to angiotensin II. These findings are of significance, in that they demonstrated direct effects of angiotensin II on expression of HO-1 by proximal tubular epithelial cells, and they complement our localization of the expression of HO-1 in rat proximal tubules in response to angiotensin II administered in vivo. These findings are in contrast to studies of expression of HO-1 in smooth muscle cells in vitro, wherein angiotensin II suppresses expression of HO-1 by smooth muscle cells [40]. To the extent that expression of HO-1 localizes the compartment within the kidney experiencing the oxidative effects of infused angiotensin II, our studies indicate that oxidant stress exerted by angiotensin II infused in vivo is borne largely by the proximal tubular compartment. Studies using the receptor blockers would be of interest in cells in culture exposed to angiotensin II so as to determine angiotensin II receptor-specific actions underlying such expression of HO-1. It is also possible that the induction of HO-1 in vivo in the renal proximal tubular compartment may reflect the functional effects of proteinuria induced by angiotensin II. Increased trafficking of protein across and through the proximal tubule can markedly influence the expression of a number of genes relevant to tissue injury, reparative responses, and inflammation. It is possible that proteinuria induced by angiotensin II may contribute to the up-regulation of HO-1 in the renal proximal tubule in vivo.

In summary, we provide evidence that angiotensin II is a potent inducer of oxidative stress in the kidney in vivo, and such oxidative stress is functionally relevant to the renal effects of angiotensin II. Moreover, angiotensin II strongly induces the expression of HO-1 in the kidney, an effect emanating, at least in part, from direct actions of angiotensin II on the renal proximal tubule.

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