Enhanced superoxide generation modulates renal function in ANG II-induced hypertensive rats

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Kopkan, Libor, Alexander Castillo, L. Gabriel Navar, and Dewan S. A. Majid. Enhanced superoxide generation modulates renal function in ANG II-induced hypertensive rats. Am J Physiol Renal Physiol 290: F80–F86, 2006. First published August 16, 2005; doi:10.1152/ajprenal.00090.2005.—This study was performed to examine the role of superoxide formation in the regulation of renal hemodynamic and excretory function and to assess its contribution in the pathogenesis of ANG II-dependent hypertension. Renal responses to acute intra-arterial infusion of the O2−scavenger tempol (50 μg·min⁻¹·100 g body wt⁻¹) with or without catalase (1,500 U·min⁻¹·100 g⁻¹; both native and polyethylene glycol-catalase), which reduces H2O2, were evaluated in anesthetized male Sprague-Dawley rats treated chronically with ANG II (65 ng/min) for 2 wk and compared with nontreated control rats. In ANG II-treated hypertensive rats, tempol caused increases in medullary (13 ± 2%), cortical (5 ± 2%), and total renal blood flow (9 ± 2%) without altering systemic arterial pressure. There were also increases in glomerular filtration rate (9 ± 2%), urine flow (17 ± 4%), and sodium excretion (26 ± 5%). However, tempol infusion in nontreated normotensive rats did not cause significant changes in any of these renal parameters. Coinfusion of catalase with tempol did not alter the responses observed with tempol alone, indicating that the observed renal responses to tempol in ANG II-treated rats were attributed to its O2−scavenging effects without the involvement of H2O2. Tempol infusion also significantly decreased 8-isoprostanate excretion in ANG II-treated rats (39 ± 6%) without changes in H2O2 excretion. However, coinfusion of catalase reduced H2O2 excretion in both ANG II-treated (41 ± 6%) and nontreated rats (28 ± 5%). These data demonstrate that enhanced generation of O2−modulates renal hemodynamic and tubular reabsorptive function, possibly leading to sodium retention and thus contributing to the pathogenesis of ANG II-induced hypertension.

superoxide; angiotensin II

ANG II is a powerful vasoconstrictor and biological hypertensinogenic agent contributing importantly to the regulation of renal function and blood pressure (21, 27, 34). Chronic administration of a low dose of ANG II, which does not cause increases in blood pressure acutely, leads to the progressive development of hypertension (5, 9, 33) and an increase in oxidative stress (27, 34). Elevated intrarenal ANG II levels cause alterations in renal function, leading to sodium retention and thus contributing to the development and maintenance of hypertension (9, 33, 38). It has been proposed that the vasoconstrictor and hypertensive effects of ANG II are due, in part, to increases in the production of superoxide (O2−) via activation of NADPH oxidase, which is an important enzymatic source of O2− in the body (2, 26).

As a highly reactive agent, O2−interacts with many endogenous substances, in particular with nitric oxide (NO), which acts as an antioxidant by reducing O2−levels (17). It is also degraded by superoxide dismutase (SOD) enzyme to form H2O2 (25). O2−oxidizes arachidonic acid nonenzymatically to generate free isoprostanes that are recognized as markers for increased endogenous O2−activity (6, 10, 17, 28). One of them, 8-isoprostanate was demonstrated to be higher in both plasma and urine samples from hypertensive rats induced by ANG II (2, 28) or endothelin (31), as well as spontaneously hypertensive rats (SHR; see Ref. 30), compared with normotensive control rats. Generally, O2−is involved in cellular signaling in a variety of tissues under normal and in pathological conditions, where its inappropriate generation may contribute to the pathophysiology of hypertension. Recent reports support a direct renal vasoconstrictor and antinatriuretic effect of O2−in vivo (15, 16, 18) as well as an effect on sodium transport in vitro (23). These results suggest an integral role of O2−in regulation of kidney function in hypertension associated with elevated levels of ANG II.

In the present study, we examined the hypothesis that ANG II-induced O2−generation influences renal vascular and tubular function, leading to sodium retention, and thus plays a role in the pathogenesis of hypertension. We evaluated the renal functional responses to an O2−scavenger, tempol (4-hydroxy-tetramethylpiperidine-1-oxyl), infused directly in the left renal artery of anesthetized male Sprague-Dawley rats treated chronically with ANG II. Normal Sprague-Dawley rats served as control animals. Tempol is a low-molecular-weight nitrooxide compound that is membrane permeable and that reduces endogenous O2−levels, as shown by many in vitro and in vivo studies (3, 15, 17, 18, 29). Because it has been suggested that administration of tempol may enhance the H2O2 level in the kidney (4, 19), we also evaluated the responses to coinfusion of catalase with tempol to delineate between the effects resulting from scavenging of O2−from those due to possible enhancement of H2O2 during administration of tempol. In these experiments, native catalase, which is poorly cell permeable, and the more cell-permeable polyethylene glycol (PEG) catalase were used to readily reduce H2O2 to water and thus minimize the action of H2O2 in the tissue (25). Intra-arterial administration of drugs was made directly in the kidney, allowing determination of their direct renal effects without alterations in blood pressure (12).

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METHODS

Animal preparation. The study was performed in male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) in accordance with the guidelines and practices established by the Tulane University Animal Care and Use Committee. After 3 days acclimation, rats (220–250 g) were randomly divided into nontreated groups and ANG II-treated groups. ANG II-treated rats were implanted with osmotic minipumps (model 2012; Alzet, Cupertino, CA) subcutaneously under anesthesia (pentobarbital sodium, 50 mg/kg ip; Sigma, St. Louis, MO). The osmotic minipumps were employed for chronic continuous infusion of a low dose of synthetic ANG II (Sigma) at a rate of 65 ng/min, which leads to the progressive development of hypertension during the course of 2 wk (2, 5, 34). In the present study, nontreated control groups were not implanted with the minipumps, since previous studies have reported that sham-operated (implanted minipump with saline) control rats do not show any differences in systemic and renal parameters compared with nonimplanted control groups (5, 36).

Systolic blood pressure (SBP) was measured one time every 2–3 days by tail-cuff plethysmography to monitor blood pressure changes during a 2-wk period before acute experiments.

At the end of 2 wk of chronic ANG II treatment, acute clearance experiments were performed to determine renal responses to tempol and catalase in anesthetized (pentobarbital sodium, 50 mg/kg ip) ANG II-infused hypertensive and nontreated normotensive rats. The right jugular vein was catheterized for intravenous administration of solutions. The right femoral artery was cannulated to allow continuous monitoring of arterial blood pressure (AcqKnowledge data acquisition system; Biopac) and blood sampling. The left kidney was exposed via a flank incision and placed in a Lucite cup, and the ureter was cannulated with a PE-10 catheter for urine collection. A tapered PE-10 catheter was inserted in the renal artery via the left femoral artery to allow intra-arterial administration of drugs directly in the kidney (12). This catheter was kept patent by a continuous infusion of heparinized isotonic saline at a rate of 5 μl/min throughout the experiment.

An ultrasonic flow probe (Transonic System) was placed on the left renal artery to measure total renal blood flow (RBF). Laser-Doppler needle flow probes (500 μm OD; Periflex 4001; Perimed) were used to measure the relative changes in cortical (CBF) and medullary (MBF) blood flow, as reported earlier (5). Zero flow was determined when the renal artery was completely occluded at the end of the experiment.

Experimental protocol. Acute experiments were conducted in the following groups of rats: A) a nontreated normotensive group with J vehicle (saline) infusion (n = 8); 2) tempol infusion (n = 9); and 3) tempol + native catalase infusion (n = 6) or B) ANG II-treated hypertensive groups receiving 4) vehicle infusion (n = 9); 5) tempol infusion (n = 9); 6) tempol + native catalase infusion (n = 6); and 7) tempol + PEG-catalase infusion (n = 4).

After 60-min stabilization, the experimental protocol was started with a 30-min control clearance period to assess baseline control values of renal hemodynamic and excretory parameters. Next the intra-arterial infusion of tempol was given for 75 min to determine renal functional responses during drug administration. After the initiation of tempol infusion, an equilibration 15-min period was allowed before two 30-min clearance experimental periods in these experiments. Tempol (Sigma Chemical) was infused at a dose of 50 μg·min⁻¹·100 g body wt⁻¹. This dose of tempol was selected based on findings in our earlier acute studies in dogs (17, 18) that showed significant reductions in urinary 8-isoprostane excretion rate (U₈ISO·V; marker for endogenous O₂⁻ activity). Catalase (both native and PEG form; Sigma Chemical) was coinfused with tempol at a rate of 1500 U·min⁻¹·100 g body wt⁻¹ (11, 24). At the midpoint of the clearance collection period, an arterial blood sample was collected from the femoral arterial cannula to measure plasma insulin and sodium concentrations.

Urine volume was measured gravimetrically. Plasma and urine sodium and potassium concentrations were determined by flame photometry, and inulin concentrations were measured colorimetrically to determine glomerular filtration rate (GFR). Renal vascular resistance (RVR) and fractional sodium excretion (FE₉Na) were calculated according to standard formulas. The enzyme immunoassay kit was used to measure urinary 8-isoprostane concentration (Assay Design, Ann Arbor, MI; see Refs. 17 and 18). Urinary H₂O₂ concentration was measured by colorimetric assay (Cayman Chemical, Ann Arbor, MI; see Refs. 13 and 18).

Data are expressed as means ± SE. Statistical comparisons between control and experimental values in the same group were conducted by paired Student’s t-test. Statistical comparisons among the groups were conducted by two-way ANOVA for repeated measurements, followed by the Newman-Keuls test. P < 0.05 was considered statistically significant.

RESULTS

Chronic infusion of a prolonged low dose of ANG II caused a slow progressive increase of SBP from 132 ± 7 to 188 ± 9 mmHg (n = 28; P < 0.001) during the 2-wk period of ANG II administration compared with normotensive nontreated rats in which SBP was not changed (134 ± 6 to 137 ± 7 mmHg; n = 23). These results are similar to those reported in previous studies (5, 33, 38).

In acute experiments in anesthetized animals, baseline values of mean arterial pressure (MAP), renal hemodynamics, and excretory parameters were assessed in all groups during the control period. Baseline MAP and RVR were significantly higher in ANG II-treated hypertensive rats and than in normotensive rats (156 ± 5 vs. 125 ± 2 mmHg and 25 ± 2 vs. 20 ± 1 mmHg·ml⁻¹·min⁻¹·g⁻¹, respectively). However, there were no significant differences in other renal parameters in either hypertensive or normotensive rats. Intra-arterial infusion of vehicle (saline) did not change MAP and renal function in either time control normotensive or hypertensive rats.

Renal hemodynamic and excretory responses to intra-arterial infusion of tempol. In normotensive rats, tempol infusion did not cause significant changes in RBF and RVR (Fig. 1). In contrast, tempol significantly increased RBF (ΔB = 2%; P < 0.05) and decreased RVR (ΔR = 8%; P < 0.05) in the ANG II-infused hypertensive groups (Fig. 1). As shown in Fig. 2, CBF and MBF were not changed significantly during infusion of tempol in normotensive rats. In hypertensive rats, tempol did not cause many changes in CBF [ΔB = 2%; P = not significant (NS); Fig. 2A] but caused a significant increase in MBF (Δ13 = 2%; P < 0.05; Fig. 2B). As shown in Fig. 3A, GFR was not significantly altered by tempol in normotensive rats; however, it was significantly increased (ΔG = 2%; P < 0.05) in the hypertensive rats during tempol infusion. Likewise, urine flow (V) responses to tempol were increased significantly (Δ7 = 4%; P < 0.05) only in the hypertensive rats but not in normotensive rats (Fig. 3B). Similar responses were also observed for sodium excretion (U₈ISO·V; Fig. 4). In normotensive rats, tempol did not significantly affect absolute or FE₉Na. However, in hypertensive rats, there were significant increases in both U₈ISO·V (Δ26 = 5%; P < 0.05) and FE₉Na (Δ19 = 4%; P < 0.05) during tempol infusion.

During administration of tempol in the renal artery, it was possible that some degree of spillover in the systemic circulation occurred. We did not measure other parameters, indicating extrarenal actions of tempol; however, there was minimal
effect of intrarenal tempol administration on systemic arterial pressure either in normotensive control (126 ± 2 to 122 ± 2 mmHg; P = NS) or in ANG II-induced hypertensive (157 ± 5 to 152 ± 5 mmHg; P = NS) rats in the present study.

Renal hemodynamic and excretory responses to intra-arterial infusion of tempol + catalase. The observed renal responses to tempol infusion alone in both hypertensive and normotensive rats were not significantly altered by coadministration of catalase in these rats (Figs. 1–4). Renal hemodynamic and excretory responses to coinfusion of native catalase with tempol were similar to those observed during PEG-catalase with tempol in ANG II-induced hypertensive rats. These responses were not significantly different from each other; therefore, data were combined for the presentations in Figs. 1–5. In ANG II-infused rats, there were decreases in RVR (Fig. 1B) and increases in RBF (Fig. 1A), MBF (Fig. 2B), GFR (Fig. 3A), V (Fig. 3B), U_{NaV}, and FE_{Na} (Fig. 4) during coadministration of tempol and catalase. CBF was not significantly increased in response to coadministration of tempol and catalase. MAP also remained unaltered during intra-arterial infusion of tempol + catalase in normotensive (123 ± 2 to 122 ± 2 mmHg; P = NS) and in hypertensive (154 ± 3 to 151 ± 2 mmHg; P = NS) animals.

Urinary excretion rate of 8-isoprostane and H_{2}O_{2} responses to tempol and tempol + catalase coinfusion. As shown in Fig. 5A, basal control values of the UIsoV were significantly higher in hypertensive rats compared with normotensive rats. Tempol infusion decreased UIsoV significantly (Δ−39 ± 6%; P < 0.01) in hypertensive rats, and the similar reductions were observed during coinfusion of tempol and catalase in hypertensive rats. There were also decreases in UIsoV (Δ−24 ± 5%; P < 0.05) in normotensive rats during tempol administration; however, the magnitude was smaller than that in hypertensive rats. Basal control urinary H_{2}O_{2} excretion rates (U_{H_{2}O_{2}}V) were not different between normotensive and hypertensive groups of rats (Fig. 5B). In both hypertensive and normotensive rats, infusion of tempol alone did not cause any significant changes...
in U_{H_2O_2}. However, coinfusion of catalase with tempol led to significant decreases in U_{H_2O_2} in both hypertensive rats (Δ−41 ± 6%; P < 0.05) and normotensive rats (Δ−28 ± 5%; P < 0.05), as shown in Fig. 5B.

**DISCUSSION**

In this present investigation, we assessed renal hemodynamic and excretory responses to tempol, a O_2 scavenging agent administered directly in the renal artery, in ANG II-induced hypertensive rats. Intra-arterial administration of tempol allowed us to evaluate its direct effect in the kidney without appreciable changes in blood pressure that are usually associated with systemic administration of tempol, as reported earlier (22, 29, 34). To our knowledge, no previous study except a recent investigation by Welch et al. (34) addressed this specific issue of determining the role of O_2 in the modulation of renal function in ANG II-induced hypertension. However, the study of Welch et al. (34) used chronic treatment of tempol, which was associated with marked reduction in arterial pressure and thus complicated the proper assessment of direct O_2 scavenging effects on renal function. In the present study, tempol was infused directly in the renal artery, which minimized its effects on systemic blood pressure and thus allowed more direct assessment of the responses to O_2 scavenging on renal hemodynamics and excretory function. It was observed that acute administration of tempol caused significant increases in RBF, GFR, V, and U_{NaV} in ANG II-induced hypertensive rats but not in normotensive control rats. Acute administration of tempol ameliorated the chronic ANG II-induced increases in U_{isoV} (marker for endogenous O_2 activity; Fig. 5A), indicating that O_2 activity is increased in these ANG II-treated rats, as reported previously (2, 28). Prolonged administration of tempol in ANG II-induced hypertensive rats was also shown to ameliorate the enhanced renal cortical NADPH oxidase activity as well as mRNA and protein expression for p22phox subunits of NADPH oxidase (34). Increases in RBF, GFR, and U_{NaV} in response to tempol in ANG II-treated rats indicate that enhanced O_2 production in these animals modulates renal function. An antinatriuretic effect of acute administration of ANG II was also shown to be partly mediated by concomitant generation of O_2 (15, 18). Thus these present data indicate that...
ANG II-induced hypertension could be, at least in part, the result of the sodium-retaining effect of enhanced O$_2$ activity. It could be argued that a possible increase in intrarenal H$_2$O$_2$ concentration during tempol administration (4, 19) influenced the observed changes in renal function in this study. Although we did not measure the tissue level of H$_2$O$_2$ in the kidney, the present results demonstrate that U$_{H_2O_2V}$ was not altered during acute tempol administration in these rats. Previous studies also reported that tempol treatment acutely in dogs (18) or chronically in rats (13) did not alter U$_{H_2O_2V}$. It was also shown that chronic tempol treatment did not alter U$_{H_2O_2V}$ in rats with normal salt intake but only in rats that were given a high-salt diet (35). However, in our earlier study (13), we have observed that high-salt intake alone in rats increased U$_{H_2O_2V}$ but not because of chronic administration of tempol. It is also known that as a modulatory agent, tempol can enhance heme proteins’ catalase-like activity, facilitating degradation of H$_2$O$_2$ (14). Supporting evidence from in vitro studies indicates that tempol decreased rather than increased H$_2$O$_2$ in renal proximal tubule cell cultures and moreover protected the cells against the cytotoxic effects of H$_2$O$_2$ (3, 8). Another point also needs to be considered that, although a modest change in renal medullary tissue concentration of H$_2$O$_2$ during tempol administration was reported earlier (4, 19), the effects of such changes in H$_2$O$_2$ on renal function are yet to be clearly defined. H$_2$O$_2$ was shown to act as a vasoconstrictor in renal medulla (4), but it has also been described as a vasodilator in renal cortical microcirculations (1). In the present study, cotreatment of catalase (both native and the PEG form) with tempol, although it caused significant reduction in U$_{H_2O_2V}$, did not lead to any differences in the responses of renal hemodynamics and excretory function caused by tempol treatment alone. Thus the present findings do not support a significant involvement of H$_2$O$_2$ in the renal responses to tempol and implicate an involvement of O$_2$ generation in the regulation of renal hemodynamics and excretory function in ANG II-induced hypertensive rats.

Tempol did not cause any significant alterations in the renal parameters in normotensive control rats (Figs. 1–4). This indicates that O$_2$ activity remains minimal in these animals. Other studies have also demonstrated that systemic administration of tempol caused MAP reduction in ANG II-infused rats but had no significant effect in normotensive control animals

Table 1. Comparison of the responses to native catalase and PEG-catalase coinfusion with tempol in ANG II-infused hypertensive rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Native Catalase + Tempol</th>
<th>PEG-Catalase + Tempol</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>Control</td>
<td>155±3</td>
</tr>
<tr>
<td>RVR, mmHg/ml·min$^{-1}$·g$^{-1}$</td>
<td>Control</td>
<td>25±1</td>
</tr>
<tr>
<td>RBF, ml·min$^{-1}$·g$^{-1}$</td>
<td>Control</td>
<td>6.36±0.28</td>
</tr>
<tr>
<td>CBF, perfusion units</td>
<td>Control</td>
<td>186±14</td>
</tr>
<tr>
<td>MFB, perfusion units</td>
<td>Control</td>
<td>47±3</td>
</tr>
<tr>
<td>GFR, ml·min$^{-1}$·g$^{-1}$</td>
<td>Control</td>
<td>0.96±0.05</td>
</tr>
<tr>
<td>V, μl·min$^{-1}$·g$^{-1}$</td>
<td>Control</td>
<td>7.68±0.43</td>
</tr>
<tr>
<td>U$_{NaV}$, μmol·min$^{-1}$·g$^{-1}$</td>
<td>Control</td>
<td>0.77±0.12</td>
</tr>
<tr>
<td>U$_{H_2O_2V}$, nmol·min$^{-1}$·g$^{-1}$</td>
<td>Control</td>
<td>2.8±0.3</td>
</tr>
<tr>
<td>FEna, %</td>
<td>Control</td>
<td>0.55±0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 rats in native catalase group and 4 rats in polyethylene glycol (PEG)-catalase group. MAP, mean arterial pressure; RVR, renal vascular resistance; RBF, renal blood flow; CBF, cortical blood flow; MFB, medullary blood flow; GFR, glomerular filtration rate; V, urine flow; U$_{NaV}$, absolute sodium excretion; FEna, fractional sodium excretion; U$_{H_2O_2V}$, urinary hydrogen peroxide excretion rate. The responses to native catalase with tempol were not significantly different from those of PEG-catalase with tempol. Because the responses were similar, data were combined for presentation in Figs. 1–5.
(22, 34). Similar results were observed in Wistar-Kyoto rats compared with SHR (30). Although O$_2^-$ is a constant product of cellular metabolism under normal conditions, its basal tissue concentration is kept to a minimal level because of efficient activity of endogenous antioxidant systems. Besides SOD and other antioxidative enzymes, endogenous NO is also known to exert a potent antioxidative effect (17). An appropriate physiological balance in oxidative status of the kidney during normal conditions is critically dependent on endogenous NO generation. As reported previously, chronic ANG II infusion significantly reduced extracellular SOD expression (2, 34); thus, the ability of enzymatic O$_2^-$ degradation may be reduced in hypertensive compared with normotensive rats. Chronic administration of tempol ameliorated the suppressed extracellular SOD and moreover normalized enhanced NADPH oxidase activity in ANG II-induced hypertension (34). These findings indicate that the O$_2^-$ level is increased in ANG II-infused hypertensive animals even though endothelial NO synthase activity may have increased in this model, as reported earlier (20).

In this study, the regional RBF responses to tempol infusion in ANG II-treated rats showed greater increases in MBF than in CBF; cumulatively, however, total RBF was increased. This indicates that concomitant generation of O$_2^-$ during chronic ANG II infusion had a greater effect on medullary circulation than on cortical circulation. Previous studies to assess regional blood flow responses to tempol also suggest higher involvement of O$_2^-$ in the renal medulla (7, 37). The diuretic and natriuretic effects of tempol in ANG II-infused hypertensive rats were modulated by increases in GFR and alterations of sodium reabsorption in the tubules. Because fractional excretion of sodium is significantly increased during tempol administration, thus causing renal vasoconstriction and antinatriuresis. The ob-

The isoprostanes: unique products of arachi-

Because it was reported that tempol exerts an inhibitory effect on sympathetic nerve activity (36), it could be argued that a neural factor was involved in the observed renal responses to tempol in the present study. However, it was also demonstrated that enhanced O$_2^-$ activity by SOD inhibition caused stimulation of renal sympathetic activity, a response that was shown to be ameliorated by tempol (32), indicating that tempol-induced inhibition of sympathetic activity could be related to its ability to scavenge O$_2^-$ in the neural tissue. In the present study, tempol infusion in the renal artery did not cause any changes in systemic arterial pressure in either normoten-

In conclusion, these data indicate that the generation of O$_2^-$ due to ANG II administration modulates renal hemodynamic and excretory function, possibly leading to sodium retention and thus contributing to the development of ANG II-dependent hypertension.

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