Enhanced superoxide generation modulates renal function in angiotensin II – induced hypertensive rats

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Short title: Superoxide and renal function in Ang II hypertension

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ABSTRACT - This study was performed to examine the role of superoxide (O$_2^-$) formation in the regulation of renal hemodynamic and excretory function and to assess its contribution in the pathogenesis of angiotensin II (Ang II) dependent form of hypertension. Renal responses to acute intra-arterial infusion of the O$_2^-$ scavenger, tempol (50 µg/min/100g BW) with or without catalase (1500 U/min/100g; both native and PEG-catalase), that reduces H$_2$O$_2$, were evaluated in anesthetized male Sprague-Dawley rats treated chronically with Ang II (65 ng/min) for 2 weeks and compared to non-treated 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 non-treated normotensive rats did not cause significant changes in any of these renal parameters. Co-infusion of catalase with tempol did not alter the responses observed with tempol alone indicated that the observed renal responses to tempol in Ang II-treated rats were attributed to its O$_2^-$ scavenging effects without the involvement of H$_2$O$_2$. Tempol infusion also significantly decreased 8-isoprostane excretion in Ang II-treated rats (39±6%) without changes in H$_2$O$_2$ excretion. However, co-infusion of catalase reduced H$_2$O$_2$ excretion in both Ang II-treated (41±6%) and non-treated rats (28±5%). These data demonstrate that enhanced generation of O$_2^-$ modulates renal hemodynamic and tubular reabsptive function possibly leading to sodium retention and thus contribute to the pathogenesis of Ang II - induced hypertension.

Key Words: superoxide, angiotensin II, hypertension, renal function.
INTRODUCTION

Angiotension 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, that does not cause increases in blood pressure acutely, leads to the progressive development of hypertension (5, 9, 33) as well as an increase in oxidative stress (27, 34). Elevated intrarenal Ang II levels cause alterations in renal function leading to sodium retention and thus contribute 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 ($O_2^-$) via activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which is an important enzymatic source of $O_2^-$ in the body (2, 26).

As a highly reactive agent, $O_2^-$ interacts with many endogenous substances, in particular with nitric oxide (NO) which acts as an antioxidant by reducing $O_2^-$ level (17). It is also degraded by superoxide dismutase (SOD) enzyme to form hydrogen peroxide ($H_2O_2$) (25). $O_2^-$ oxidizes arachidonic acid nonenzymatically to generate free isoprostanes which are recognized as markers for increased endogenous $O_2^-$ activity (6, 10, 17, 28). One of them, 8-isoprostane 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) (30) compared to normotensive control rats. Generally, $O_2^-$ is involved in cellular signaling in a variety of tissues under
normal as well as 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 O$_2^-$ \textit{in vivo} (15, 16, 18) as well as an effect on sodium transport \textit{in vitro} (23). These results suggest an integral role of O$_2^-$ 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 O$_2^-$ 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 O$_2^-$ scavenger, tempol (4-hydroxy-tetramethylpiperidime-1-oxyl), infused directly into 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, which is membrane permeable that reduces endogenous O$_2^-$ levels as shown by many \textit{in-vitro} and \textit{in-vivo} studies (3, 15, 17, 18, 29). Because it has been suggested that administration of tempol may enhance hydrogen peroxide (H$_2$O$_2$) level in the kidney (4, 19), we also evaluated the responses to co-infusion of catalase with tempol to delineate between the effects due to scavenging of O$_2^-$ from those due to possible enhancement of H$_2$O$_2$ during administration of tempol. In these experiments, native catalase which is poorly cell permeable as well as more cell permeable polyethylene glycol (PEG) catalase were used to readily reduce H$_2$O$_2$ to water and thus minimizes the action of H$_2$O$_2$ in the tissue (25). Intra-arterial administration of drugs was made
directly into the kidney allowing determination of their direct renal effects without alterations in blood pressure (12).
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 a non-treated groups and the Ang II - treated groups. Ang II treated rats were implanted with osmotic minipumps (model 2002; Alzet, Cupertino, CA) subcutaneously under anesthesia (pentobarbital sodium, 50 mg/kg ip; Sigma Chemical, St. Louis, MO). The osmotic minipumps were employed for chronic continuous infusion of a low dose of synthetic Ang II (Sigma Chemical, St. Louis, MO) at a rate of 65 ng/min which leads to the progressive development of hypertension during the course of two weeks (2, 5, 34). In the present study, non-treated control groups were not implanted with the minipumps, as previous studies have reported that sham operated (implanted mini-pump with saline) control rats do not show any differences in systemic and renal parameters compared to non-implanted control groups (5, 36). Systolic blood pressure (SBP) was measured once every 2-3 days by tail-cuff plethysmography, to monitor blood pressure changes during a two week period, prior to acute experiments.

At the end of two weeks 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 non-treated 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 into the renal artery via the left femoral artery to allow intra-arterial administration of drugs directly into the kidney (12). This catheter was kept patent by a continuous infusion of heparinized isotonic saline at a rate of 5μl/min throughout the experiment.

An ultrasonic flow probe (Transonic system, Ithaca, USA) was placed on the left renal artery to measure total renal blood flow (RBF). Laser-Doppler needle flow probes (500 μm O.D., Periflex 4001, Perimed, Sweden) were used to measure the relative changes in cortical (CBF) and medullary blood flow (MBF), 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) non-treated normotensive group:
   1. vehicle (saline) infusion (n=8),
   2. tempol infusion (n=9)
   3. tempol + native catalase co-infusion (n=6);

b) Ang II - treated hypertensive group:
   4. vehicle infusion (n=9),
   5. tempol infusion (n=9),
   6. tempol + native catalase co-infusion (n=6)
   7. tempol + PEG - catalase co-infusion (n=4).

After 60-minute stabilization, the experimental protocol was started with a 30-minute control clearance period to assess baseline control values of renal hemodynamic and excretory parameters. Then the intra-arterial infusion of tempol was given for 75 minutes to determine renal functional responses during drug administration. After the initiation of tempol infusion, an equilibration 15-minute period was allowed prior to two 30-minute clearance experimental periods in these experiments. Tempol (Sigma Chemical Co.) was infused at a dose 50 µg/min/100 g BW. 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 (UISOV; marker for endogenous O2− activity). Catalase (both native and PEG form; Sigma Chemical Co.) was co-infused with tempol at a rate of 1500 U/min/100 g BW (11, 24). At the mid-point of clearance
collection period, an arterial blood sample was collected from femoral arterial cannula to measure plasma Inulin and sodium concentration.

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 ($\text{FE}_\text{Na}$) were calculated according to standard formulas. Enzyme immunoassay kit was used to measure urinary 8-isoprostane concentration (Assay Design Inc; Ann Arbor, MI, USA) (17, 18). Urinary $\text{H}_2\text{O}_2$ concentration was measured by colorimetric assay (Cayman Chemical; Ann Arbor, MI, USA) (13, 18).

Data are expressed as means ± SE. Statistical comparisons between control and experimental values in the same group were conducted by paired Student t-test. Statistical comparisons among the groups were conducted by two-way ANOVA for repeated measurements, followed by Newman-Keuls test. P value ≤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 \(132 \pm 7\) mmHg to \(188 \pm 9\) mmHg (n=28; \(P<0.001\)) during the two week period of Ang II administration as compared with normotensive non-treated rats where SBP was not changed (\(134 \pm 6\) to \(137 \pm 7\) 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) and 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 \((156 \pm 5\) vs. \(125 \pm 2\) mmHg and \(25 \pm 2\) vs. \(20 \pm 1\) mmHg/ml.min.g, 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 both time-control normotensive and 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 (\(\Delta 9\pm2%;\ P<0.05\)) and decreased RVR (\(\Delta 8\pm1%;\ P<0.05\)) 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, although tempol did not cause much changes in CBF (\(\Delta 5\pm2\%\); 
P=n.s. Fig. 2A), but caused significant increase in MBF (\(\Delta 13\pm2\%; P<0.05\); Fig. 
2B). As illustrated in Fig. 3A, GFR was not significantly altered by tempol in 
normotensive rats, however it was significantly increased (\(\Delta 9\pm2\%; P<0.05\)) in 
the hypertensive rats during tempol infusion. Likewise, urine flow (V) responses 
to tempol was increased significantly (\(\Delta 17\pm4\%; P<0.05\)) only in the hypertensive 
rats but not in normotensive rats (Fig. 3B). Similar responses were also observed 
in sodium excretion (\(U_{\text{Na}}V\); Fig. 4). In normotensive rats, tempol did not 
significantly affect absolute as well as fractional sodium excretion (\(FE_{\text{Na}}\)). 
However in hypertensive rats, there were significant increases in both 
\(U_{\text{Na}}V\) (\(\Delta 26\pm5\%; P<0.05\)) and \(FE_{\text{Na}}\) (\(\Delta 19\pm4\%; P<0.05\)) during tempol infusion.

During administration of tempol into the renal artery, it was possible that 
some degree of spillover into 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 124 ± 3 mmHg; P=n.s.) or in 
Ang II induced hypertensive rats (157 ± 5 to 152 ± 5 mmHg; P=n.s.) 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 co-
administration of catalase in these rats (Fig. 1-4). Renal hemodynamic and 
excretory responses to co-infusion of native catalase with tempol were similar to
those observed during PEG-catalase with tempol in the experiments conducted in hypertensive rats. Table 1 provides the comparison of the responses to native catalase and PEG-catalase given with tempol in Ang II-induced hypertensive rats. These responses were not significantly different to each other, therefore data were combined for the presentations in figures. 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 F_{E_{Na}} (Fig. 4) during co-administration of tempol and catalase. CBF was not seen significantly increased in response to co-administration of tempol and catalase. MAP also remained unaltered during intra-arterial infusion of tempol + catalase in normotensive (123 ± 2 to 122 ± 2 mmHg; P=n.s.) as well as in hypertensive animals (154 ± 3 to 151 ± 2 mmHg; P=n.s.).

**Urinary excretion rate of 8-isoprostane and H_{2}O_{2} responses to tempol and tempol + catalase co-infusion.** As illustrated in Fig. 5A, basal control values of urinary excretion rate of 8-isoprostane (U_{ISOV}) were significantly higher in hypertensive rats compared with normotensive rats. Tempol infusion decreased U_{ISOV} significantly (Δ -39±6%; P<0.01) in hypertensive rats and the similar reductions were observed during co-infusion of tempol and catalase in hypertensive rats. There were also decreases in U_{ISOV} (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_{H2O2V}) 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_{H2O2V}. However, co-infusion of catalase with tempol led to significant decreases in U_{H2O2V} in both hypertensive
rats ($\Delta -41\pm 6\%$; $P<0.05$) and normotensive rats ($\Delta -28\pm 5\%$; $P<0.05$), 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 into 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 into the renal artery; that 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, urine flow and sodium excretion 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 p22^{phox} subunits of NADPH oxidase (34). Increases in RBF, GFR and urinary sodium excretion 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, due to the sodium retaining effect of enhanced O_2^- activity.

It could be argued that a possible increase in intrarenal H_2O_2 concentration during tempol administration (4, 19) influenced the observed changes in renal function in this study. Although we did not measure tissue level of H_2O_2 in the kidney, the present results demonstrate that U_{H_2O_2} 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_2}. It was also shown that chronic tempol treatment did not alter U_{H_2O_2} 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_2} but not due to chronic administration of tempol. It is to be emphasized here that tempol has also been described as a modulatory agent of the hemeproteins catalase-like activity facilitating degradation of H_2O_2 (14). Supporting evidence from in-vitro studies indicates that tempol decreased rather than increased H_2O_2 in renal proximal tubule cell cultures and moreover protected the cells against the cytotoxic effects of H_2O_2 (3, 8). Another point also needs to be considered that although a modest change in
renal medullary tissue concentration of $\text{H}_2\text{O}_2$ during tempol administration was reported earlier (4, 19), the effects of such changes in $\text{H}_2\text{O}_2$ on renal function are yet to be clearly defined. $\text{H}_2\text{O}_2$ 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, co-treatment of catalase (both native and PEG form) with tempol, though it caused significant reduction in $U_{\text{H}_2\text{O}_2}\text{V}$, 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 $\text{H}_2\text{O}_2$ in the renal responses to tempol and implicate an involvement of $\text{O}_2^-$ 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 (Fig. 1-4). This indicates that $\text{O}_2^-$ 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 (22, 34). Similar results were observed in Wistar-Kyoto rats compared with SHR (30). Although $\text{O}_2^-$ is a constant product of cellular metabolism under normal condition, its basal tissue concentration is kept to a minimal level due to 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) and 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 \( O_2^- \) 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 renal blood flow 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 as well as alterations of sodium reabsorption in the tubules. As fractional excretion of sodium is significantly increased during tempol administration in Ang II - treated rats, it is also notable that enhanced \( O_2^- \) activity directly modulates tubular reabsorptive function as has been reported previously in \textit{in-vitro} (23) and \textit{in-vivo} studies (15, 16). \( O_2^- \) may exert its direct action in the kidney (14) or indirectly by reducing NO bioavailability (5, 17, 29) thus causing renal vasoconstriction and antinatriuresis. The observed renal responses to tempol in hypertensive rats could be due either to reduction of \( O_2^- \) activity or an increase in the bioavailability of NO or both. In an
earlier study in dogs (18), we observed that renal responses to acute administration of Ang II was ameliorated by tempol infusion both in intact conditions as well as under conditions of NO synthase inhibition. In isolated thick ascending limb preparations, tempol decreased NaCl absorption without increasing NO levels; that suggests the direct effect of $\text{O}_2^-$ modulated NaCl absorption in thick ascending limb is independent of NO (23). Thus, these results provide further evidence that an increase in $\text{O}_2^-$ generation modulates renal hemodynamics and excretory function during chronic administration of Ang II.

As 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 $\text{O}_2^-$ activity by SOD inhibition caused stimulation of renal sympathetic activity, a response which was shown to be ameliorated by tempol (32), indicating that tempol induced inhibition of sympathetic activity could be related to its ability to scavenge $\text{O}_2^-$ in the neural tissue. In the present study, tempol infusion into the renal artery did not cause any changes in systemic arterial pressure in either normotensive or hypertensive rats indicating a minimal neural involvement in the observed responses to tempol. Moreover, the renal artery was isolated from surrounding tissue by severing the visible renal nerve fibres and thus the kidney was mostly denervated during these acute experiments. In our earlier studies, where a denervated kidney preparation was conducted in dogs, tempol was also shown to induce changes in renal function during treatment with Ang II and NO inhibition (17, 18). The vasodilator and natriuretic responses to tempol may indicate an involvement of natriuretic factors
in these responses. However, marked changes in circulating factors such as atrial natriuretic peptide induced by tempol administration were not expected as there was no indication of any changes in circulatory volume during tempol administration. Moreover, tempol did not cause any effects in normotensive rats, thus a marked involvement of sympathetic activity and circulating natriuretic factors seems unlikely in the renal responses to tempol observed in the present study.

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 contributes to the development of Ang II-dependent hypertension.
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GRANT

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Author's conflict of interest: NONE
REFERENCES


FIGURES LEGENDS

**Figure 1.** Renal blood flow (RBF) (A) and renal vascular resistance (RVR) (B) responses to intra-arterial infusion of tempol in normotensive (○; n=9) and Ang II - treated hypertensive (●; n=9) rats as well as co-infusion of tempol + catalase in normotensive (▽; n=6) and hypertensive (▲; n=10) rats. *, P<0.05 vs. corresponding control values; #, P<0.05 vs. values in normotensive rats.

**Figure 2.** Cortical blood flow (CBF) (A) and medullary blood flow (MBF) (B) responses to intra-arterial infusion of tempol in normotensive (○; n=9) and hypertensive (●; n=9) rats as well as co-infusion of tempol + catalase in normotensive (▽; n=6) and hypertensive (▲; n=10) rats. *, P<0.05 vs. corresponding control values.

**Figure 3.** Glomerular filtration rate (GFR) (A) and urine flow (V) (B) responses to intra-arterial infusion of tempol in normotensive (○; n=9) and hypertensive (●; n=9) rats as well as co-infusion of tempol + catalase in normotensive (▽; n=6) and hypertensive (▲; n=10) rats. *, P<0.05 vs. corresponding control values.
**Figure 4.** Absolute sodium excretion (UNaV) (A) and fractional sodium excretion (FENa) (B) responses to intra-arterial infusion of tempol in normotensive (○; n=9) and hypertensive (♦; n=9) rats as well as co-infusion of tempol + catalase in normotensive (▼; n=6) and hypertensive (▲; n=10) rats. *, P<0.05 vs. corresponding control values.

**Figure 5.** Urinary 8-isoprostane excretion rate (U_{IsoV}) (A) and urinary H_{2}O_{2} excretion rate (U_{H2O2V}) (B) responses to intra-arterial infusion of tempol in normotensive (○; n=9) and hypertensive (♦; n=9) rats as well as co-infusion of tempol + catalase in normotensive (▼; n=6) and hypertensive (▲; n=6,10) rats. *, P<0.05 vs. corresponding control values; #, P<0.05 vs. values in normotensive rats.
Table 1: Comparison of the responses to native catalase (n=6) and PEG-catalase (n=4) co-infusion with tempol in Ang II - infused hypertensive rats.

<table>
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<th>Parameter</th>
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<th>experimental</th>
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<td></td>
<td>nat. catalase + tempol</td>
<td>PEG-catalase + tempol</td>
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<td>MAP (mmHg)</td>
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<td>MBF (PU)</td>
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<td>U_{Na}V (µmol/min.g)</td>
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<td>U_{H2O2}V (nmol/min.g)</td>
<td>2.8±0.3</td>
<td>1.6±0.3</td>
<td>-44±9</td>
<td>2.4±0.2</td>
<td>1.5±0.1</td>
<td>-39±6</td>
</tr>
</tbody>
</table>

Values are mean ± SE. MAP, mean arterial pressure; RVR, renal vascular resistance; RBF, renal blood flow; CBF, cortical blood flow; MBF, medullary blood flow; GFR, glomerular filtration rate; V, urine flow; U_{Na}V, absolute sodium excretion; F_{ENa}, fractional sodium excretion, U_{H2O2}V, urinary hydrogen peroxide excretion rate. The responses to native catalase with tempol were not significantly different than those to PEG-catalase with tempol. As the responses were similar, the data were combined for the presentation in figures.
Figure 1

A

![Graph showing RBF (ml/min.g) comparison between control and experimental groups.]

B

![Graph showing RVR (mmHg/ml.min.g) comparison between control and experimental groups.]
Figure 2

A

B

Control experimental

CBF (PU)

MBF (PU)

control experimental
Figure 3

A

GFR (ml/min.g)

B

V (µl/min.g)
Figure 4

A

B

$U_{Na,V}$ (µmol/min.g)

$FE_{Na}$ (µmol/min.g)

control experimental

control experimental

* *
Figure 5

A

B