Enhanced Superoxide Activity Modulates Renal Function in NO-Deficient Hypertensive Rats

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Abstract—An enhancement of superoxide (O$_2^-$) activity was shown to contribute to the development of hypertension induced by NO deficiency. To better understand the mechanistic role of O$_2^-$ in this NO-deficient hypertension, we evaluated the renal responses to acute intraarterial administration of an O$_2^-$ scavenger, tempol (50 μg/min per 100 g of body weight) in anesthetized male Sprague-Dawley rats treated with NO synthase inhibitor nitro-L-arginine methyl ester (15 mg/kg per day in drinking water, n=7) for 4 weeks, which caused increases in mean arterial pressure (146±3 versus 124±2 mm Hg) compared with normotensive control rats (n=6). Hypertensive rats had higher renal vascular resistance (29±2 versus 20±1 mm Hg/mL per minute per gram), as well as lower renal blood flow (5.2±0.3 versus 6.3±0.2 mL/min per gram; cortical blood flow, 153±13 versus 191±8 perfusion units; medullary blood flow, 43±2 versus 51±3 perfusion units) and glomerular filtration rate (0.69±0.04 versus 0.90±0.05 mL/min per gram) without a significant difference in urinary sodium excretion (0.81±0.07 versus 0.86±0.12 μmol/min per gram) compared with normotensive rats. Urinary 8-isoprostane excretion rate (6.8±0.7 versus 4.5±0.3 pg/min per gram) was higher in hypertensive than normotensive rats. Intraarterial infusion of tempol did not alter renal function in normotensive rats. However, tempol significantly decreased renal vascular resistance by 12±2% and urinary 8-isoprostane excretion rate by 24±4% and increased renal blood flow by 10±2%, cortical blood flow by 9±2%, medullary blood flow by 15±6%, glomerular filtration rate by 11±3%, and urinary sodium excretion by 19±5% in hypertensive rats. These data indicate that enhanced O$_2^-$ activity modulates renal hemodynamics and excretory function during reduced NO production and, thus, contributes to the pathophysiology of the NO-deficient form of hypertension. (Hypertension. 2006;47[part 2]:568-572.)

Key Words: nitric oxide ■ oxidative stress ■ hypertension, renal

An imbalance between the production and the degradation of reactive oxygen species such as superoxide anion (O$_2^-$) leads to the condition termed as “oxidative stress.” It has been suggested that oxidative stress is involved in the pathophysiology of many forms of hypertension.¹⁻³ Treatment with an O$_2^-$ scavenging agent, tempol (4-hydroxy-tetramethylpyperidime-1-oxyl), significantly reduces blood pressure in different hypertensive models⁴⁻⁵ indicating that O$_2^-$ plays a role in the development of hypertension. Recently, we also demonstrated that chronic treatment with tempol attenuated the development of hypertension and salt sensitivity in rats induced by chronic inhibition of NO synthase (NOS) in rats.⁶ These results indicate that an enhanced O$_2^-$ generation is involved in the pathogenesis of an NO-deficient form of hypertension. However, the exact mechanistic role of O$_2^-$ in mediating the salt sensitivity and hypertension in the condition of NO deficiency has not yet been clearly defined.

NO acts as an endogenous antioxidative agent by reacting with O$_2^-$ generated in the living tissues, thus it provides a protective function against the action of O$_2^-$ in many organs, including the kidney.⁶⁻⁷ Previous studies have indicated that an increasing accumulation of O$_2^-$ in biological tissues can occur in the condition of NO deficiency that can lead to alterations in organ function.⁵⁻⁸ In an earlier study in dogs, we observed that tempol treatment before NOS inhibition did not cause any functional changes in the kidney but caused diuresis and natriuresis during NOS inhibition indicating that an enhancement of O$_2^-$ activity because of NO deficiency influences renal tubular function.⁷ Previous studies also provided evidence that an exaggerated impairment of kidney function occurred in hypertensive animals during NO inhibition.⁹⁻¹¹ The findings in these studies support the notion that an interaction between NO and O$_2^-$ has a role in regulating normal function in the kidney, an imbalance of which would lead to the development of hypertension.

In the present study, we examined the hypothesis that increased O$_2^-$ activity because of chronic NOS inhibition influences renal vascular and tubular function that compromises the ability of the kidney to excrete sodium appropriately and, thus, plays a role in the pathogenesis of the NO-deficient form of hypertension. We evaluated the renal
functional responses to tempol, infused directly into the left renal artery of anesthetized male Sprague-Dawley rats treated chronically with the NOS inhibitor nitro-L-arginine methyl ester (L-NAME) for 4 weeks. Normal Sprague-Dawley rats served as control animals. To our knowledge, no previous study addressed this specific issue of determining the role of O$_2^\cdot$ in modulating renal function in L-NAME–induced hypertensive rats. As we reported previously, as demonstrated by our previous study, 12 intraarterial administration of tempol provides a more direct assessment of the responses to O$_2^\cdot$ scavenging on renal hemodynamics and excretory function without appreciable changes in blood pressure that are usually associated with systemic administration of tempol. 13,14

Methods

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 (250 to 270 g) were randomly divided into the nontreated groups (n = 6) and the L-NAME–treated groups (n = 7). L-NAME (Sigma) at a dose of 15 mg/kg per day was given in drinking water for a 4-week experimental period.

At the end of 4 weeks of chronic L-NAME treatment, acute clearance experiments were performed to determine renal responses to tempol in anesthetized (pentobarbital sodium, Sigma; 50 mg/kg IP) L-NAME–treated hypertensive and nontreated normotensive rats as described previously. 15 The right jugular vein was catheterized for intravenous administration of solutions. The cannula introduced into the right femoral artery was connected with the AcqKnowledge data acquisition system Biopac to allow continuous monitoring of arterial blood pressure. 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 polyethylene tube PE-10 (OD, 0.61 mm) catheter, which was tapered ~40% to 50%, was inserted into the left femoral artery from the aorta via the left femoral artery to allow intraarterial administration of drugs directly into the kidney at a rate of 5 μL/min. 12 This procedure of inserting the tapered catheter usually does not compromise the renal blood flow (RBF) measurements, because it was observed in pilot experiments that there was no significant alteration of baseline RBF before and after the catheter insertion.

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

After a 60-minute stabilization, the experimental protocol was started with two 30-minute control clearance periods to assess the baseline control values of renal hemodynamic and excretory parameters. Then, the intraarterial infusion of tempol was given for 75 minutes to determine the renal functional responses during drug administration. After the initiation of tempol infusion, a 15-minute equilibration period was allowed before two 30-minute clearance experimental periods in these experiments. Tempol (Sigma) was infused at a dose of 50 μg/min per 100 g of body weight. This dose of tempol was selected based on findings in our earlier acute studies in rats 12 and dogs 7,16 that showed significant reductions in the urinary 8-isoprostane excretion rate (U8-iso; marker for endogenous O$_2^\cdot$ activity). At the midpoint of the clearance collection period, an arterial blood sample was collected from the femoral arterial cannula to measure plasma inulin and sodium concentration.

Urine volume was measured gravimetrically. Plasma and urinary sodium 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 (FENa) were calculated according to standard formulas. An enzyme immunoassay kit was used to measure urinary 8-isoprostane concentration (Cayman Chemical). 4,6

Data are expressed as mean ± 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 2-way ANOVA for repeated measurements, followed by Newman–Keuls test. P value ≤ 0.05 was considered statistically significant.

Results

In acute experiments, baseline values of mean arterial pressure and renal hemodynamics and excretory parameters were assessed in anesthetized animals during the control period. Baseline mean arterial pressure was significantly higher in L-NAME–treated hypertensive rats than in normotensive rats (146 ± 2 versus 124 ± 2 mm Hg). Baseline RBF (Figure 1A) was lower, and baseline RVR (Figure 1B) was higher in L-NAME–treated hypertensive rats compared with normotensive rats. Comparisons of the baseline values of CBF and MBF between hypertensive and normotensive rats were not made, because these were measured as relative blood flow using laser-Doppler flowmetry and expressed as the percentage of the control value (taken as 100%; Figure 2 A and 2B). However, GFR (Figure 3A) measured by the inulin clearance technique was lower compared with normotensive control rats. There were no significant differences in urine flow (V; Figure 3B) and sodium excretion (Figure 4) between hypertensive or normotensive rats. However, it was observed that the baseline plasma sodium concentration was slightly but significantly higher in L-NAME–treated hypertensive rats.
than in normotensive control rats (148.0 ± 0.4 versus 146.1 ± 0.6 mmol/L; \( P < 0.03 \)).

Figures 1 to 4 illustrate the effects of acute tempol administration on the renal function in both hypertensive and normotensive rats. Tempol administration did not significantly alter renal hemodynamic and excretory function in normotensive rats as we demonstrated previously.\(^6\) However, in l-NAME–treated hypertensive rats, tempol infusion caused a significant increase in RBF (Δ10 ± 2%; \( P < 0.05 \); Figure 1A) and decreased RVR (Δ12 ± 2%; \( P < 0.05 \); Figure 1B). There were also increases in regional blood flow to the renal cortex (CBF; Δ9 ± 2%; \( P < 0.05 \); Figure 2A) and to the renal medulla (MBF; Δ15 ± 6%; \( P < 0.05 \); Figure 2B) during tempol infusion in l-NAME–treated rats. As illustrated in Figure 3A, GFR was also significantly increased by tempol in l-NAME–treated hypertensive rats (Δ11 ± 2%; \( P < 0.05 \)). Although the increases in urine flow during tempol infusion were of borderline significance statistically (Δ9 ± 4%; \( P < 0.07 \); Figure 3B), the absolute urine sodium excretion (Δ19 ± 5%; \( P < 0.05 \)) and FENa (Δ11 ± 4%; \( P < 0.05 \)) were significantly increased as illustrated in Figure 4A and 4B. During administration of tempol in the renal artery, there was a minimal effect on systemic arterial pressure either in normotensive control (124 ± 2 to 122 ± 3 mm Hg; \( P \) value not significant) or in l-NAME–treated hypertensive rats (146 ± 2 to 143 ± 3 mm Hg; \( P \) value not significant).

As illustrated in Figure 5, baseline control values of \( U_{ISO}V \) were significantly higher in hypertensive rats compared with normotensive rats. Tempol administration decreased the \( U_{ISO}V \) as reported previously.\(^6\)\(^7\)\(^8\) In hypertensive rats, tempol infusion decreased \( U_{ISO}V \) by 24 ± 4% (\( P < 0.01 \); Figure 5). There was also a decrease in \( U_{ISO}V \) by 11 ± 3% (\( P < 0.05 \); Figure 5) in normotensive rats during tempol administration; however, the magnitude was smaller than that in hypertensive rats.

**Discussion**

In the present study, it has been demonstrated that acute treatment with an \( O_2^- \) scavenger, tempol, increases the RBF, GFR, and urinary excretion rate of sodium in chronic l-NAME–induced hypertensive rats but not in normotensive control rats. Because tempol was infused directly into the renal artery that minimized its effect on systemic blood pressure,\(^7\)\(^12\)\(^16\) a more direct assessment of \( O_2^- \) scavenging effects on renal hemodynamics and excretory function was possible in these experiments. The results clearly indicate that an enhancement of \( O_2^- \) activity modulates renal hemodynamics and excretory function during NOS inhibition in rats. This assessment is additionally supported by the fact that chronic l-NAME treatment in these rats caused higher \( U_{ISO}V \) (marker for endogenous \( O_2^- \) activity; Figure 5) compared with nontreated animals, which was ameliorated by such acute intrarenal administration of tempol. Earlier, we also demonstrated that chronic treatment with tempol attenuated the development of salt sensitivity and hypertension in rats that were given l-NAME for a 4-week period.\(^6\) Taken together, these results demonstrate that enhanced \( O_2^- \) activity in the condition of NO deficiency modulates renal hemodynamics and excretory function, which contributes to the development of salt sensitivity and hypertension induced by chronic NOS inhibition. Previous studies also suggested that the hyperten-
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Figure 4. Absolute sodium excretion (UNaV; A) and FE\textsubscript{Na} (B) responses to intraarterial infusion of tempol in normotensive (○; n=6) and l-NAME-treated hypertensive (▲; n=7) rats. *P<0.05 vs corresponding control values.

Figure 5. $U_{iso}V$ responses to intraarterial infusion of tempol in normotensive (○; n=6) and l-NAME-treated hypertensive (▲; n=7) rats. *P<0.05 vs corresponding control values; **P<0.01 vs corresponding control values; #P<0.05 vs values in normotensive rats.

sorption observed in Dahl salt-sensitive rats was linked to enhanced $O_2^-$ activity.17

In rats, both acute and chronic administration of an NOS inhibitor causes renal vasoconstriction and a decrease in GFR as reported previously.9,11,18,19 The present investigation also confirms that chronic l-NAME treatment induces decreases in total and regional blood flow to the kidney, as well as GFR, as compared with the values obtained from nontreated control animals. As observed with chronic tempol treatment in our earlier study,6 acute intrarenal infusion of tempol in l-NAME–hypertensive rats in the present study also increased GFR. This finding indicates that an enhancement of $O_2^-$ modulates glomerular hemodynamics in the condition of NO deficiency, possibly by altering pregglomerular vascular resistance as suggested earlier.20 Because tempol infusion caused increases in absolute, as well as fractional excretion of sodium in l-NAME–treated rats but not in intact animals (Figure 4), it is reasonable to speculate that enhanced $O_2^-$ activity because of NOS inhibition directly modulates tubular reabsorptive function. A similar observation was also reported in our earlier studies using dogs7 in which intrarenal administration of tempol caused natriuretic response during NO inhibition but not in intact condition. The exact tubular segment in which $O_2^-$ exerts its effect was not possible to determine from our in vivo experiments; however, a previous in vitro study by Ortiz and Garvin21 reported that $O_2^-$ enhances sodium reabsorption in a thick ascending limb of the loop of Henley. Chronic administration of tempol also showed increases in sodium excretion, as well as attenuation of the hypertensive responses in rats treated with l-NAME for 4 weeks.6 In the present study, we also observed that mean baseline plasma sodium concentration was significantly higher in l-NAME–treated rats than in nontreated control rats. These findings may indicate that a degree of sodium retention has occurred in rats because of l-NAME treatment. Collectively, these results support the hypothesis that sodium retention resulting from oxidative stress–induced enhancement in tubular sodium reabsorption plays a mechanistic role in the development of an NO-deficient form of hypertension. However, additional comprehensive studies may be required to confirm this hypothesis, because our present study was not designed to assess the sodium balance or volume status in these rats treated chronically with or without l-NAME.

Although $O_2^-$ is a constant product of cellular metabolism under normal condition, its basal tissue concentration is kept to a minimal level because of efficient activity of endogenous antioxidant systems. It is increasingly evident that endogenous NO also exerts a potent antioxidative effect and that an appropriate physiological balance in the oxidative status of the kidney during normal condition is critically dependent on endogenous NO generation.7,8 We have demonstrated earlier that RBF, GFR, and excretory function in anesthetized dogs remain appreciably well protected during acute treatment with an inhibitor of $O_2^-$ dismutase enzyme in intact animals but not in NO-blocked dogs.22 It was also shown that acute treatment with the $O_2^-$ scavenger tempol increased urine flow and sodium excretion in NO-blocked dogs but not in intact animals.7 In the present study, tempol treatment did not cause any appreciable changes in renal parameters in normotensive control rats but only in rats subjected to chronic NO deficiency. These findings clearly indicate a powerful antioxidative function of endogenous NO that protects critical organ function from the adverse effects of continually released endogenous $O_2^-$ both in physiology, as well as in pathophysiology of many disease processes. It is conceivable that in any condition of NO deficiency, tissue $O_2^-$ concentration would increase because of a lack of NO-mediated antioxidative action. Supporting this notion, previous studies also demonstrated that NOS inhibition enhances vascular $O_2^-$ release in rats.23
mice,\textsuperscript{24} and humans.\textsuperscript{25} Although we did not measure directly the O$_2^-$ level in the present study, we observed that U$_{150}$V increased in l-NAME–treated rats, which was significantly reduced by acute tempol infusion. In our previous study in dogs,\textsuperscript{7} we also observed an increase in U$_{150}$V during acute NOS inhibition in the kidney that was ameliorated by coadministration of tempol. In conclusion, these data demonstrate that the enhanced O$_2^-$ activity because of chronic NOS inhibition contributes to the impairment of renal function that compromises the ability of the kidney to excrete sodium appropriately and, thus plays a role in the pathogenesis of the NO-deficient form of hypertension.

Perspectives

The findings of this present study, as well as our earlier observations,\textsuperscript{6,7,16} emphasize an important role of the interaction between O$_2^-$ and NO in the regulation of renal function and blood pressure. These observations suggest that decreased NO availability can also induce an imbalance between oxidative and antioxidative mechanism in living tissues, which is involved in many pathophysiologic processes in the body. Thus, additional emphasis should be given in future experimental studies to determine the interactive role of O$_2^-$ and NO in the regulation of many organ functions to increase our understanding of physiological as well as pathophysiological processes of many cardiovascular and renal diseases that are commonly associated with NO deficiency.

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References


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