Normaliztion of Blood Pressure and Renal Vascular Resistance in SHR With a Membrane-Permeable Superoxide Dismutase Mimetic
Role of Nitric Oxide

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Abstract—Superoxide radical ($O_2^-$) is increased in the vessel wall of spontaneously hypertensive rats (SHR) where its blockade potentiates endothelium-dependent vasodilatation. The purpose of this study was to determine the role of $O_2^-$ in the hypertension and renal vasoconstriction of SHR and its interaction with nitric oxide (NO). Baseline mean arterial pressure (MAP) and renal vascular resistance were markedly elevated in SHR (n=6) compared with Wistar-Kyoto rats (WKY; n=6) (145±4 versus 118±4 mm Hg, $P<0.05$, and 24±3 versus 17±1 mm Hg·mL$^{-1}$·min$^{-1}$, respectively; $P<0.05$). The stable membrane-permeable superoxide dismutase mimetic 4-hydroxy-2,2,6,6-tetramethyl piperidine-1-oxyl (tempol; 72 μmol/kg IV) normalized MAP (103±9 versus 96±6 mm Hg for SHR and WKY, respectively) and RVR (17±2 versus 15±1 mm Hg·mL$^{-1}$·min$^{-1}$) of SHR. The MAP of SHR was more sensitive and responsive to graded infusions of tempol (0, 1.8, 18, 180, and 1800 μmol·kg$^{-1}$·h$^{-1}$ IV) than that of WKY. To determine whether $O_2^-$ increases MAP by inactivation of NO, its synthesis was blocked in SHR with $N^G$-nitro-$L$-arginine methyl ester (L-NAME, 11 μmol·kg$^{-1}$·min$^{-1}$ IV, n=6). Whereas tempol alone significantly reduced MAP by 32% (184±12 to 121±18 mm Hg, $P<0.05$, n=6), L-NAME infusion abolished the MAP response to tempol (187±8 to 186±4 mm Hg, n=5). In contrast, tempol did reduce MAP of SHR (188±7 to 161±7 mm Hg, $P<0.05$) where MAP was elevated by norepinephrine (31 nmol·kg$^{-1}$·min$^{-1}$ IV, n=6). Finally, to determine the longer-term effect of $O_2^-$, tempol (1.5 mmol·kg$^{-1}$·d$^{-1}$ IP) was given for 7 days. Tempol had no effect on MAP in WKY (96±1 to 97±1 mm Hg, n=7) but significantly decreased MAP in SHR (133±2 to 120±3 mm Hg, $P<0.05$, n=7). These data implicate $O_2^-$ in the hypertension of SHR in vivo. The antihypertensive action of tempol depends on NO synthesis presumably because $O_2^-$ inactivates NO and thus diminishes its vasodilatory actions. (Hypertension. 1998;32:59-64.)

Key Words: free radicals ■ superoxide dismutase ■ nitric oxide ■ antioxidants ■ tempol ■ blood pressure

Hypertension has been associated with low levels of endogenous antioxidants such as vitamin C. Clinical studies show that intravenous infusion of vitamin C or other antioxidants significantly reduces blood pressure in hypertensive patients.2-3 The SHR, a model of essential hypertension, is characterized by increased oxidative stress. Using fluorescence microscopy in vivo, Suzuki et al4 showed that mesenteric arterioles of the SHR have increased $O_2^-$ production. Furthermore, Auch-Schwelk et al5 demonstrated that aortic rings prepared from SHR are more sensitive to $O_2^-$ than are those from WKY. The enhanced aortic contractions to $O_2^-$ in SHR were blocked by the $O_2^-$ scavenger SOD. However, previous studies investigating the importance of endogenously generated oxygen radicals in the regulation of blood pressure in SHR have shown modest or scant results.6-8 This may relate to the properties of the $O_2^-$ scavenger administered: some forms such as allopurinol also simultaneously produce $O_2^-$, other forms such as native SOD lack membrane permeability, and CuZn SOD is inactivated by divalent ions found intracellularly.

The mechanism for the vasodilatory actions of $O_2^-$ scavengers seen in in vitro studies remains unclear. Several vascular beds of SHR have impaired endothelium-dependent vasodilatation.9-11 Gryglewski et al12 showed that $O_2^-$ reacts with NO to form peroxynitrite, thereby effectively depleting NO in vascular endothelial cells. Furthermore, Rubanyi and Vanhoucke13 demonstrated that $O_2^-$ inactivates endothelium-derived relaxing factor in coronary artery rings. Scavenging of $O_2^-$ enhances endothelium-dependent vasodilatation and increases NO release from mesenteric arterioles15 and endothelial cells16 in SHR.

Although there is significant in vitro evidence suggesting that $O_2^-$ contributes to increased systemic vascular tone in the SHR, the role of $O_2^-$ in the increased RVR and MAP of SHR in vivo remains unclear. The purpose of this study was to determine the role of $O_2^-$ in the steady-state regulation of
RVR and MAP and to determine the role of NO in the MAP response to scavenging of \( \text{O}_2^- \) in SHR. We used the stable, metal-independent, membrane-permeable SOD mimetic tempol, which has been shown to be a stable spin trap for \( \text{O}_2^- \) and to reduce \( \text{O}_2^- \)-related injury in ischemia/reperfusion, inflammation, and radiation.

**Methods**

Groups of male SHR and WKY (200 to 300 g) were maintained on tap water and standard chow (Harlan-Teklad Inc). Protocols were approved by the Institutional Animal Care and Use Committee of Georgetown University Medical Center and were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, as well as the guidelines of the Animal Welfare Act. All rats were divided into four groups. In group 1, renal hemodynamics and MAP during bolus intravenous injection of tempol were compared in anesthetized SHR and WKY. In group 2, the MAP during constant intravenous infusion of tempol was compared in anesthetized SHR and WKY. In group 3, the role of NO in the MAP response to constant infusion of tempol in SHR was investigated. In group 4, the longer-term MAP response to tempol was compared in SHR and WKY.

**Group 1: Renal Hemodynamics and MAP During Tempol Bolus**

WKY (n=6) and SHR (n=6) were anesthetized with thiobutabarbital (100 mg/kg IP, Inactin, Research Biochemicals International) and maintained at 37°C on a servo-controlled heated rodent operating table. A tracheostomy was performed with polyethylene PE-240 tubing. Intravenous infusion of 1% albumin dissolved in 0.154 mol/L NaCl solution was infused at 2 mL/h IV to maintain a euvoletic state. A midline incision was made, and the left renal artery and connected to a transit-time blood flowmeter (1RB, Transonic Systems Inc). We have previously shown that this method of measuring real-time changes in RBF is valid in the rat. MAP was measured before and after 7 days of tempol. Rats were administered at a rate of 1.5 mmol·kg\(^{-1}\)·h\(^{-1}\).

**Results**

**Figure 1.** MAP during baseline conditions (Basal) and during bolus injection of tempol (24 and 72 \( \mu \)mol/kg IV) in anesthetized WKY (\( \bullet \), n=6) and SHR (\( \circ \), n=6). *P<0.05 vs Basal; †P<0.05 vs WKY.

Figure 1 shows the MAP during baseline conditions and infusion of tempol at 24 and 72 \( \mu \)mol/kg in WKY and SHR. Baseline MAP was significantly elevated in SHR compared with WKY (145±4 versus 118±3 mm Hg, respectively; \( P<0.05 \)). Low-dose tempol (24 \( \mu \)mol/kg IV) had no effect in either the WKY (114±5 mm Hg) or SHR (147±4 mm Hg). However, higher-dose tempol normalized the MAP of the SHR to the level of WKY. Tempol (72 \( \mu \)mol/kg IV) significantly (\( P<0.05 \)) decreased MAP by 11% in WKY (96±6 mm Hg) and by 28% in SHR (104±9 mm Hg).

**Figure 2.** depicts renal hemodynamics during basal conditions and infusion of tempol at 24 and 72 \( \mu \)mol/kg in WKY and SHR. Baseline RBF was similar between groups (WKY, 6.6±0.8 mL/min) and was not affected during tempol (WKY, 6.6±0.7; SHR, 6.7±0.8 mL/min). In contrast, baseline RVR was significantly increased in SHR compared with WKY (24±3 versus 17±1 mm Hg·mL\(^{-1}\)·min\(^{-1}\), respectively; \( P<0.05 \)). Low-dose tempol had no effect on
RVR in either group (WKY, 17±6; SHR, 24±6 mm Hg \cdot mL^{-1} \cdot min^{-1}). However, higher-dose tempol normalized the RVR of the SHR to the level of WKY. Tempol at 72 μmol/kg significantly (P<0.05) decreased RVR by 29% in SHR (17±2 mm Hg \cdot mL^{-1} \cdot min^{-1}) while having a minimal effect in WKY (15±1 mm Hg \cdot mL^{-1} \cdot min^{-1}).

**Group 2: MAP During Constant Tempol Infusion**

Figure 3 illustrates the dose-response relationship between tempol at 1.8, 18, 180, 1800 μmol \cdot kg^{-1} \cdot h^{-1} and MAP in WKY and SHR. Baseline MAP was again significantly (P<0.05) elevated in the SHR (166±7 mm Hg) compared with WKY (121±4 mm Hg). Tempol dose-dependently decreased MAP in WKY and SHR, with SHR having a greater sensitivity and responsiveness to tempol infusion. The highest dose of tempol (1800 μmol \cdot kg^{-1} \cdot h^{-1}) normalized the MAP of SHR (72±10 mm Hg) to the level of WKY (71±3 mm Hg).

**Group 3: MAP During Constant Tempol Infusion—Effect of NO Synthesis Blockade**

Figure 4 illustrates the percent change in MAP in SHR pretreated with isotonic saline vehicle (2 mL/min IV) or the NO synthesis inhibitor L-NAME (11 μmol \cdot kg^{-1} \cdot min^{-1} IV). As in the previous group, infusion of tempol (180 μmol \cdot kg^{-1} \cdot min^{-1}) for 30 minutes significantly decreased MAP by 32% in SHR (121±17 mm Hg, P<0.05). In marked contrast, the NO synthesis inhibitor L-NAME abolished the MAP response to tempol. Twenty minutes of L-NAME infusion alone increased MAP by 18% from 158±11 to 187±8 mm Hg, and MAP remained unchanged during tempol infusion (186±4 mm Hg). Time control studies in a separate group of SHR showed that MAP remained steady during L-NAME infusion (change in MAP at 50 minutes, 0.3±3.3%; NS). To investigate whether the failure of tempol to lower MAP in L-NAME–infused rats was a consequence of the severe vasoconstriction and hypertension, the protocol was repeated in SHR infused with norepinephrine (31 nmol \cdot kg^{-1} \cdot min^{-1}) in place of L-NAME. Norepinephrine increased MAP by 15% from 164±4 to 188±7 mm Hg. This was
similar to the increase with L-NAME. However, tempol significantly decreased MAP by 14% (161 ± 7 mm Hg, \( P < 0.05 \)) in SHR infused with norepinephrine. Time-control studies in a separate group of SHR showed that MAP remained steady during norepinephrine infusion (change in MAP at 50 minutes, 2.0 ± 0.0%; NS).

**Group 4: Longer-term Effect of Tempol on MAP**

Figure 5 depicts the change in MAP after 7 days of tempol administration in WKY and SHR. Baseline MAP was significantly (\( P < 0.05 \)) elevated in SHR (133 ± 2 mm Hg) compared with WKY (96 ± 1 mm Hg) but was lower than in previous groups, probably because a different anesthesia was used. After 7 days of tempol (1.5 mmol · kg\(^{-1} \) · d\(^{-1} \) IP), there was no change in MAP of the WKY (97 ± 1 mm Hg). In contrast, tempol significantly reduced MAP of the SHR by 10% to 120 ± 4 mm Hg (\( P < 0.05 \)).

**Discussion**

Both the blood pressure and the renal vasculature of the SHR have an increased responsiveness and sensitivity to the vasodilatation produced by scavenging of \( \text{O}_2^- \). Tempol, a membrane-permeable SOD mimetic, normalized MAP and RVR of SHR. Because the antihypertensive response was blocked by NO synthesis inhibition, it must depend on NO. \( \text{O}_2^- \) also appears to be important in the longer-term control of blood pressure in SHR. These results suggest that SHR have increased \( \text{O}_2^- \) activity that contributes to their hypertension. In these studies, the effect of tempol on RVR may have been secondary to its effect on blood pressure with engagement of renal autoregulation.

\( \text{O}_2^- \) is generated and acts both extracellularly and intracellularly, where it can have harmful effects including lipid peroxidation, protein aggregation, and DNA destruction. Previous investigators have used scavengers of \( \text{O}_2^- \) to reduce inflammation, atherosclerosis, and ischemia/reperfusion injury. Because native SOD has limited membrane permeability and has proved to be disappointing in preventing adverse effects of \( \text{O}_2^- \) or in reducing blood pressure in vivo, alternative agents with SOD-like activity have been investigated. However, some SOD mimetics such as CuZn SOD are metal dependent and can become ineffective intracellularly because of metal-ligand dissociation. Therefore, compounds with SOD-like activity having low molecular weight, biological stability, no toxicity, and membrane permeability are preferred for use in vivo. Mitchell et al have shown that tempol is a low-molecular-weight, stable SOD mimetic that is metal independent and cell membrane permeable. Tempol does not act as a catalase mimetic or alter hydrogen peroxide concentration, and tempol does not bind NO or produce \( \text{O}_2^- \). These findings suggest that tempol is specific to the superoxide radical. Tempol prevents \( \text{O}_2^- \)-induced damage during inflammation, radiation, and cardiac reperfusion injury, and protects cardiac myocytes from ischemic damage.

Previous studies investigating the short-term actions of \( \text{O}_2^- \) on blood pressure in SHR demonstrated that bolus injection of a xanthine oxidase inhibitor to block the formation of \( \text{O}_2^- \) from xanthine or CuZn SOD acutely decreased MAP in the SHR; however, results for WKY were not reported. Therefore, we compared the effect of scavenging \( \text{O}_2^- \) on MAP in SHR to their genetic control WKY. We show that acute tempol administration normalized MAP and RVR in SHR to the level of WKY. In addition, 7 days of tempol administration reduced MAP by 10% in SHR, whereas it had no effect in WKY. This last result confirms the finding that long-term administration of another \( \text{O}_2^- \) scavenger, vitamin C, reduces blood pressure in SHR. Our data show that \( \text{O}_2^- \) is increased selectively in SHR compared with the normotensive control, which did not have any response to 7 days of tempol administration. The data also show that acute tempol administration had a stronger blood pressure-lowering effect than 7-day treatment. The disparity between the results may be due to the route and dose of administration of tempol. Previous investigators showed that intraperitoneal tempol administration in mice yields a peak blood concentration (600 μg/mL) in 5 to 10 minutes, after which the concentration rapidly declines with a half-life of 2 hours. We chose the 7-day dose of tempol because higher doses given intraperitoneally increase mortality.

Earlier studies have established a role for \( \text{O}_2^- \) in the aorta and mesenteric arteries of SHR. However, the kidneys play an important role in the development and maintenance of hypertension. Tempol vasodilated the renal vasculature in SHR more than in WKY. Under control conditions, RVR was significantly elevated in SHR, and tempol normalized RVR in SHR to the level of WKY. Because tempol reduced MAP without changing RBF, renal vasodilation was inferred. The RVR response to tempol may be a result of RBF autoregulation. Whether tempol directly or indirectly decreases RVR in SHR remains to be further elucidated.

The mechanism of the selective reduction in blood pressure by scavenging of \( \text{O}_2^- \) is unclear. One possible explanation may be that \( \text{O}_2^- \) can inactivate NO and thereby blunt the vasodilatory pathway. Several studies have shown that blockade of NO causes hypertension in animal models and humans. SHR have reduced endothelium-dependent vasodilation in several vascular beds, including the kidney, that has been ascribed in part to increased NO degradation by \( \text{O}_2^- \). Tschudi et al demonstrated that the defective release of
NO from mesenteric arterioles of SHR could be normalized after SOD. Grunfeld et al\textsuperscript{16} showed that endothelial cells cultured from aorta of stroke-prone SHR had an apparent decrease in NO release that was fully reversed by SOD and therefore presumably represented enhanced NO degradation by O$_2^-$. In their study, blockade of SOD enhanced endothelium-dependent relaxation of the aorta of SHR to a greater extent than in WKY. Our data demonstrate that intravenous infusion of tempol decreases MAP by 32% in SHR and that this response is blocked in SHR pretreated with the NO synthase inhibitor L-NAME. To ensure that the negative response to tempol during L-NAME was not merely due to an increase in systemic vascular resistance and blood pressure, we examined the MAP response to tempol in SHR infused with norepinephrine. In SHR pretreated with norepinephrine, which produced a similar increase in MAP, tempol reduced MAP by 14%. Previous investigators have shown that catecholamines, including norepinephrine, have antioxidant properties.\textsuperscript{36} Because norepinephrine is an antioxidant, the addition of another antioxidant would be less effective. For this reason, tempol may have been less effective in lowering MAP in SHR pretreated with norepinephrine (14%) than in normal SHR (32%). Overall, these data suggest that NO plays an important role in mediating the antihypertensive actions of O$_2^-$. There are several possible mechanisms by which NO mediates the antihypertensive actions of tempol. First, tempol may directly donate NO. This possible mechanism has been proven incorrect because tempol does not decompose to NO.\textsuperscript{37} Second, scavenging of O$_2^-$ increases the half-life of NO. Gryglewski et al\textsuperscript{13} showed that O$_2^-$ is important in the breakdown of NO to peroxynitrite, and Rubanyi and Vanhoutte\textsuperscript{14} demonstrated that O$_2^-$ inactivates NO in coronary artery rings. There are several possible sources of O$_2^-$. including xanthine oxidase, NADPH oxidase, incomplete electron transport, and even brain NO synthase.\textsuperscript{30} The source of O$_2^-$ in our study remains unclear. However, because previous studies suggest a role for O$_2^-$ released from the vasculature in SHR, brain NO synthase does not appear to be the major source. As a result of the powerful interaction between O$_2^-$ and NO, tempol may prolong the half-life of NO and thus allow it to exert a more powerful vasodilatory action. Finally, by blocking the formation of peroxynitrite, tempol may inhibit the production of vasoconstrictor endoperoxides that are stimulated by peroxynitrite in macrophages.\textsuperscript{38}

In summary, short- and longer-term administration of the stable, membrane-permeable SOD mimetic tempol significantly reduces MAP in SHR to a greater extent than in WKY. Tempol also significantly reduced RVR in SHR. Whether this decrease was due to a direct action on renal vessels or an autoregulatory response to changes in renal perfusion pressure remains to be determined. Overall, this is the first study showing that scavenging of O$_2^-$ both extracellularly and intracellularly with a membrane-permeable SOD mimetic normalizes the RVR and MAP of SHR. The antihypertensive actions of tempol are dependent on NO. Whether scavenging of O$_2^-$ decreases MAP through direct or indirect action on the l-arginine/NO pathway requires further investigation.


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