Superoxide scavenging attenuates renal responses to ANG II during nitric oxide synthase inhibition in anesthetized dogs

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Majid, Dewan S. A., Akira Nishiyama, Keith E. Jackson, and Alexander Castillo. Superoxide scavenging attenuates renal responses to ANG II during nitric oxide synthase inhibition in anesthetized dogs. Am J Physiol Renal Physiol 288: F412–F419, 2005. First published October 5, 2004; doi:10.1152/ajprenal.00294.2004.—To assess the role of superoxide (O2−) and nitric oxide (NO) interaction in mediating the renal actions of ANG II, we examined the renal responses to acute infusion of ANG II (0.5 ng·kg−1·min−1) before and during administration of a superoxide dismutase mimetic, tempol (0.5 mg·kg−1·min−1), in the presence or absence of NO synthase inhibitor, nitro-L-arginine (NLA; 50 μg·kg−1·min−1), in anesthetized dogs pretreated with enalaprilat (33 μg·kg−1·min−1). In one group of dogs (n = 7), ANG II infusion before tempol infusion caused decreases of 24 ± 4% in renal blood flow (RBF), 55 ± 7% in urine flow (V), and 53 ± 8% in urinary sodium excretion (UNaV) with a slight decrease in glomerular filtration rate (GFR; −7.8 ± 3.4%). Tempol infusion alone did not cause significant alterations in RBF, GFR, V, or UNaV; however, ANG II in the presence of tempol caused a smaller degree of decreases in RBF (−12 ± 2%), in V (−16 ± 5%), and in UNaV (−27 ± 10%) with a slight increase in GFR (6.6 ± 2.8%) than the responses observed before tempol. In another group of NLA-treated dogs (n = 6), tempol infusion also caused significant attenuation in the ANG II–induced responses on RBF (−13 ± 3% vs. −22 ± 7%), GFR (−19 ± 5% vs. −33 ± 3%), V (−15 ± 12% vs. −28 ± 4%), and UNaV (−11 ± 14% vs. −32 ± 7%). These data demonstrate that renal responses to ANG II are partly mediated by O2− generation and its interaction with NO. The sodium-retaining effect of ANG II is greatly influenced by O2− generation, particularly in the condition of NO deficiency.

CELLULAR METABOLISM OF MOLECULAR oxygen results in the production of reactive oxygen species (ROS), such as superoxide anion (O2−; see Ref. 6). An inappropriate generation of ROS is a feature of many disease processes, including high blood pressure-related vasculopathy or nephropathy (2, 32). The ROS generation in the kidney could play a critical role in the pathogenesis of hypertensive disease, since this contributes to many derangements in renal function (28, 32). The link between ROS and the development of hypertension has been proposed, since it was observed that hypertensive patients possess higher levels of some ROS components and lower levels of the antioxidant vitamin C in the plasma compared with normotensive subjects (13). O2−, a common ROS component, has been shown to be associated with development of hypertension (13, 15, 37, 39). Furthermore, administration of the superoxide dismutase (SOD) mimetic tempol (4-hydroxy-2,2,6,6-tetramethyl piperidinoxyl) causes reductions in blood pressure in spontaneously hypertensive rats (SHR) and ANG II-induced hypertensive rats (25, 30, 31).

Although a role for O2− has been suggested in ANG II–induced hypertension, it is not yet clear how much it can contribute to the acute vasoconstrictor or antinatriuretic effects of ANG II in the kidney. O2− has been implicated in various studies to be a potential mediator of ANG II–induced intracellular signaling pathways (7, 35). It has been shown that both O2− and nitric oxide (NO) can be formed endogenously by the action of ANG II (12, 27, 33). Recent studies have also implicated a potential interaction between O2− and NO in the regulation of renal vascular and tubular function (20, 39). We have reported earlier that enhancement of O2− activity by the administration of an SOD inhibitor in the kidney results in vasoconstriction and sodium retention (20). These renal effects of SOD inhibition were augmented during blockade of NO formation (20). These findings indicate that O2− can exert direct vasoconstriction and tubular effects independently of its interaction with NO in the kidney. In a recent study in rats, renal effects of acute administration of ANG II have shown to be partially attenuated with a SOD mimetic or an inhibitor of NADPH oxidase (18). Because O2− generation can also reduce NO bioavailability, it is possible that the acute actions of ANG II are modulated by O2− and NO interactions in the kidney. However, the possible impacts of this O2−–NO interaction on the renal vascular and tubular actions of ANG II are yet to be determined.

We hypothesized that the renal vasoconstrictor and antinatriuretic responses to ANG II are influenced by O2− generation and that NO plays a renoprotective role in balancing the adverse effects of O2−. To examine this hypothesis, the present investigation was designed to evaluate the role of O2− generation and its possible interaction with NO on ANG II–induced changes in renal hemodynamics and excretory function. Accordingly, renal responses to acute infusion of ANG II were evaluated before and during administration of a SOD mimetic, tempol, in anesthetized dogs pretreated with or without an NO synthase (NOS) inhibitor, nitro-L-arginine (NLA; see Refs. 19–21). All of these experiments in dogs were carried out during continuous infusion of the angiotensin-converting enzyme (ACE) inhibitor enalaprilat (33 μg·kg−1·min−1; see Ref. 24) to minimize changes in endogenous levels of ANG II.

METHODS

These experiments were performed in accordance with the guidelines and practices established by the Tulane University Animal Care and Use Committee. Mongrel dogs of either gender (12–23 kg body weight) were anesthetized with sodium pentobarbital (50 mg·kg−1·i.v.) and ventilated with a Harvard respirator (Harvard Apparatus, Holliston, MA) under halothane anesthesia. After intubation, the body temperature of each dog was maintained at 37°C by a heating pad and water vapor. After the experiment, dogs were euthanized with sodium pentobarbital (100 mg·kg−1·i.v.) and perfused with saline followed by 10% formalin saline. After perfusion, each kidney was removed, and the renal vasculature was dissected from the renal capsule. Each kidney was weighed and sliced transversely into 1-cm segments. The paraffin-embedded tissue sections were stained with hematoxylin-eosin, and the histologic slides were used to determine renal tissue thickness. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
to achieve a sodium-replete state, these dogs were given a supplemental amount of sodium chloride (1.5 g·kg body wt\(^{-1}\)·day\(^{-1}\)) added to the normal laboratory ration for 3 days (20, 21). They were anesthetized with pentobarbital sodium (30 mg/kg iv), with additional amounts given as needed to maintain an appropriate level of anesthesia. The surgical preparation of the animals and basic experimental techniques are identical to those previously described (19–21). Briefly, a cuffed endotracheal tube was inserted in the trachea and connected to an artificial respirator, which was set at a rate of 18 strokes/min with a stroke volume of 15 ml/kg body wt. Body temperature was maintained at a near-constant level (~100°F) with an electric heating pad.

Systemic arterial pressure was measured from a catheter placed in the abdominal aorta and connected to a square-wave flowmeter (Carolina Medical Electronics). A curved 23-gauge needle cannula was inserted in the renal artery distal to the flow probe and was connected to a pressure transducer for measurement of renal arterial pressure. Another catheter was also connected to this needle cannula for continuous infusion of heparinized saline or drug solutions at a rate of 0.4 ml/min.

The experiments were conducted in two groups of dogs that were given continuous infusion of enalaprilat (33 μg·kg\(^{-1}\)·min\(^{-1}\); see Ref. 24). Enalaprilat is the active metabolite of the prodrug enalapril maleate. This is a rapidly acting drug when administered intravenously. It has been observed that intra-arterial administration of enalaprilat in anesthetized dogs shows its maximum effects on RBF within 10 min of administration (24). In the first group (n = 7), renal responses to infusion of ANG II (0.5 mg·kg\(^{-1}\)·min\(^{-1}\)) were evaluated before and during infusion of tempol (0.5 mg·kg\(^{-1}\)·min\(^{-1}\)). This dose of ANG II was chosen from preliminary experiments that showed an ~25% decrease in basal RBF. To assess the changes during interventions with other drugs, this range of vasoconstrictor responses to ANG II was considered reasonable. The dose of tempol was used previously in our laboratory and was shown to completely reduce the enhanced 8-isoprostanate levels (marker for oxidative stress) during NOS inhibition in dogs (21). Initially, we tested by infusing this dose of tempol intra-arterially in anesthetized dogs that showed a reduction of ~60% in urinary excretion rate of 8-isoprostanate (U\(_{8\text{ISO}}\)) during a 30-min infusion period (unpublished observation). Such reduction in 8-isoprostanate excretion was similar to what was reported earlier in SHR with the use of a similar dose that was effective in normalizing the blood pressure in SHR (30, 31). In the second group of dogs (n = 6), renal responses to ANG II infusion were evaluated before and during NOS inhibition by NLA (50 μg·kg\(^{-1}\)·min\(^{-1}\); see Refs. 19–21) and then during combined infusion of NLA plus tempol.

**Experimental protocols in group 1.** After completion of surgery and a 45-min stabilization period, the experimental protocol began with two consecutive 10-min control urine collections with arterial blood samples (2 ml) collected at the midpoint of each collection period. Next, a continuous infusion of enalaprilat (24) was initiated intra-arterially for the whole duration of the experimental period. Thus, in the present protocol, the responses to enalaprilat were evaluated during two 10-min collection periods after a 10-min stabilization period. At the end of two 10-min collection periods, ANG II was added to the infusion line in which enalaprilat is continuously given throughout the whole protocol period. After 10 min of the initiation of ANG II infusion, two more 10-min urine samples were collected. Next, ANG II infusion was stopped, and 10 min were allowed for stabilization before collections of two more 10-min urine samples (control collections before tempol administration). Tempol was then added to the infusion line. After 10 min of tempol infusion, two 10-min urine collections were made. Then, ANG II infusion was added to the infusion line with tempol, and two more collections of urine were made after 10 min of the initiation of combined tempol plus NLA infusion. In three additional dogs, ANG II infusion was repeated with a gap period of 1 h without coadministration of tempol to examine the ANG II responsiveness resulting from its repeated administration (time control experiments).

**Experimental protocols in group 2.** The protocol began with two consecutive 10-min control collections, followed by a continuous intra-arterial infusion of enalaprilat (22) for the whole duration of the experimental period. After 10 min of the initiation of enalaprilat infusion, two additional 10-min urine samples were collected. Next, an intra-arterial infusion of ANG II was initiated, and renal clearances were evaluated as stated earlier in the group 1 protocol. After the cessation of ANG II infusion, an intra-arterial infusion of NLA was initiated and continued for the rest of the period. After 30 min of the initiation of NLA infusion, two 10-min urine collections were made (19, 20). In all previous studies in dogs with NLA in our laboratory, a 30-min stabilization period was considered, as this period was seen sufficient to achieve a maximal effect of NLA on RBF. After collection of two 10-min urine samples during NLA infusion period, ANG II was added to the infusion line, and renal clearances were evaluated during ANG II infusion, as stated above. ANG II infusion was then stopped, and 10 min were allowed for recovery of the renal parameters. Next, tempol was added to the NLA infusion line (21). After 10

### Table 1. Responses to ANG II before and during tempol infusion (n = 7)

<table>
<thead>
<tr>
<th></th>
<th>Control Period</th>
<th>Enal</th>
<th>Enal + ANG II</th>
<th>Enal</th>
<th>Enal + Tempol</th>
<th>Enal + Tempol + ANG II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic arterial pressure, mmHg</td>
<td>121 ± 5</td>
<td>113 ± 6</td>
<td>115 ± 6</td>
<td>115 ± 7</td>
<td>119 ± 6</td>
<td>122 ± 6</td>
</tr>
<tr>
<td>Renal blood flow, ml·min(^{-1})·g(^{-1})</td>
<td>4.2 ± 0.4</td>
<td>4.9 ± 0.4*</td>
<td>3.8 ± 0.4†</td>
<td>4.5 ± 0.5</td>
<td>4.2 ± 0.3</td>
<td>3.7 ± 0.4‡</td>
</tr>
<tr>
<td>Renal vascular resistance, mmHg·ml(^{-1})·min(^{-1})·g(^{-1})</td>
<td>30.6 ± 3.7</td>
<td>24.8 ± 3.5*</td>
<td>33.4 ± 6.1*</td>
<td>27.1 ± 4.1</td>
<td>30.1 ± 2.8</td>
<td>37.8 ± 5.7†</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml·min(^{-1})·g(^{-1})</td>
<td>0.85 ± 0.01</td>
<td>0.93 ± 0.02*</td>
<td>0.86 ± 0.02†</td>
<td>0.93 ± 0.04</td>
<td>0.87 ± 0.06</td>
<td>0.92 ± 0.05†</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.34 ± 0.02</td>
<td>0.32 ± 0.02</td>
<td>0.42 ± 0.03†</td>
<td>0.38 ± 0.03</td>
<td>0.39 ± 0.03</td>
<td>0.49 ± 0.05‡</td>
</tr>
<tr>
<td>Urine flow, μl·min(^{-1})·g(^{-1})</td>
<td>11.5 ± 1.2</td>
<td>22.5 ± 3.7*</td>
<td>8.7 ± 1.0†</td>
<td>12.1 ± 1.0</td>
<td>12.8 ± 2.5</td>
<td>10.4 ± 1.5</td>
</tr>
<tr>
<td>Sodium excretion, μmol·min(^{-1})·g(^{-1})</td>
<td>2.9 ± 0.3</td>
<td>5.1 ± 0.6*</td>
<td>2.2 ± 0.1†</td>
<td>3.0 ± 0.1</td>
<td>3.1 ± 0.4</td>
<td>2.2 ± 0.1‡</td>
</tr>
<tr>
<td>Fractional excretion of sodium, %</td>
<td>2.4 ± 0.3</td>
<td>3.9 ± 0.6*</td>
<td>1.7 ± 0.2*</td>
<td>2.4 ± 0.3</td>
<td>2.5 ± 0.4</td>
<td>1.7 ± 0.2‡</td>
</tr>
<tr>
<td>Potassium excretion, μmol·min(^{-1})·g(^{-1})</td>
<td>0.45 ± 0.05</td>
<td>0.49 ± 0.06</td>
<td>0.40 ± 0.04</td>
<td>0.44 ± 0.07</td>
<td>0.48 ± 0.06</td>
<td>0.45 ± 0.04</td>
</tr>
<tr>
<td>Nitrate/nitrite excretion, nmol·min(^{-1})·g(^{-1})</td>
<td>0.90 ± 0.08</td>
<td>1.40 ± 0.27*</td>
<td>0.69 ± 0.05†</td>
<td>0.91 ± 0.11</td>
<td>0.96 ± 0.17</td>
<td>0.80 ± 0.15</td>
</tr>
</tbody>
</table>

Values are means ± SE. Enal, enalaprilat infusion. Control values before tempol infusion appear in column 4. P < 0.05 vs. control period (*); vs. Enal (Pre-ANG II period) †); and vs. Enal + tempol period (‡).

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min of the initiation of tempol infusion, two 10-min urine samples were collected. Thereafter, ANG II infusion was initiated in the presence of NLA plus tempol. After 10 min of stabilization, two 10-min urine collections were made during ANG II infusion.

Sodium, potassium, and inulin concentrations in plasma and urine were determined as previously described (19–21). Urinary concentrations of 8-isoprostanone and NO metabolites nitrate/nitrite were determined using an enzyme immunoassay kit (Assay Design; see Ref. 21). Urinary concentration of H2O2 was also measured with an H2O2 assay kit (Cayman Chemical). Because the measurement of urinary 8-isoprostanone and H2O2 was not considered in the initial design of the protocol for group 1 dogs, urine was not collected methodically and, thus, was not considered for analysis in group 1. Values are reported as means ± SE. Statistical comparisons of differences in the responses were conducted with either ANOVA (in case of absolute changes) or Student’s paired t-test (in case of percent changes). Differences in the mean values were deemed significant at P ≤ 0.05.

RESULTS

Effects of tempol on renal responses to ANG II (group 1). The summarized results obtained from seven dogs are shown in Table 1. Intrarenal ACE inhibition with enalaprilat resulted in changes in RBF, renal vascular resistance (RVR), and other renal parameters, as reported earlier (19, 24). It was noted that the enalaprilat treatment caused a small but significant (P < 0.05) increase in GFR in these dogs. ANG II infusion in enalaprilat-treated dogs caused increases in RVR (P < 0.05) and decreases (P < 0.05) in RBF, urine flow (V), and urinary sodium excretion (UNaV), with a minimal decrease in glomerular filtration rate (GFR). Table 1 provides the absolute values and Figs. 1–4 show both the absolute (A) and percent (B) changes in RBF, GFR, V, and UNaV responses, respectively, observed during ANG II infusion in these seven dogs. ANG II infusion in these anesthetized dog preparations caused increases in filtration fraction, as reported previously (29). During tempol treatment, a similar increment in filtration fraction resulting from ANG II was also observed (Table 1). After cessation of ANG II infusion for nearly 30 min, the RBF and excretory function showed a partial recovery of the control values before ANG II infusion; the reason for such incomplete recovery was not clear. In a few of these dogs, extension of the recovery period beyond 40–50 min also did not restore these baselines completely. However, the administration of tempol in these enalaprilat-treated dogs did not cause any significant renal parameter changes (Table 1). Interestingly, the renal vasoconstrictor and the antinatriuretic responses to ANG II were markedly attenuated during infusion of tempol. Urinary nitrate/nitrite excretion rate (UNOXV) increased significantly (P < 0.05) during enalaprilat treatment (Table 1). UNOXV decreased significantly (P < 0.05) in response to ANG II before but not during tempol infusion (Table 1).

Effects of tempol on renal responses to ANG II in NOS-inhibited dogs (group 2). The summarized results in absolute values obtained from six dogs are shown in Table 2, and the changes (both absolute and percent) in the responses to ANG II are shown in Figs. 5–8. Intra-arterial infusion of the ACE inhibitor enalaprilat resulted in similar responses, as observed in the group 1 dogs. As reported previously (19–21), intra-arterial infusion of NLA resulted in a significant (P < 0.05) increase in RVR and reductions in RBF, V, and UNaV without significant change in GFR. ANG II infusion in these dogs

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Fig. 1. Effect of ANG II (0.5 ng·kg−1·min−1) infusion on renal blood flow (RBF) before and during administration of tempol (0.5 mg·kg−1·min−1) in dogs (n = 7). A: responses in absolute changes; the units for baseline values are ml·min−1·g−1. B: responses in percent changes. P < 0.05 vs. baseline values (*) and between values obtained before and during tempol infusion (#).

Fig. 2. Effect of ANG II (0.5 ng·kg−1·min−1) infusion on glomerular filtration rate (GFR) before and during administration of tempol (0.5 mg·kg·min−1) in dogs (n = 7). A: responses in absolute changes; the units for baseline values are ml·min−1·g−1. B: responses in percent changes. P < 0.05 vs. baseline values (*) and between values obtained before and during tempol infusion (#).

Fig. 3. Effect of ANG II (0.5 ng·kg−1·min−1) infusion on urine flow (V) before and during administration of tempol (0.5 mg·kg·min−1) in dogs (n = 7). A: responses in absolute changes; the units for baseline values are µl·min−1·g−1. B: responses in percent changes. P < 0.05 vs. baseline values (*) and between values obtained before and during tempol infusion (#).
before NOS inhibition by NLA also resulted in similar responses, as in group 1. There were somewhat similar changes in RBF and in RVR due to ANG II infusion before and during NLA administration. However, ANG II caused a marked reduction (P < 0.001) in GFR during NLA infusion (Fig. 6), as reported previously in conscious dogs (1). ANG II infusion during NLA treatment showed decreases (P < 0.05) in V and UNaV (Figs. 6 and 7) but not in fractional excretion of sodium during NLA infusion was significantly (P < 0.05) attenuated during coadministration of tempol during ANG II infusion before infusion of NLA. As expected, NLA infusion decreased (P < 0.05) UNOXV compared with the values during enalaprilat treatment or the control period and did not change significantly during the combination of NLA and tempol infusion, as reported previously (21). UNOXV did not change significantly in response to ANG II during NLA or NLA plus tempol infusion periods. The urinary excretion rate of H2O2 (n = 5) showed a wide variation among the dogs, and the changes in response to the administration of enalaprilat, ANG II, tempol, or NLA were not significantly different from each other. However, the values of urinary excretion of H2O2 observed in the present study were within the range that was reported earlier in human urine (17).

Time-dependent changes in the renal responses to ANG II during tempol/NLA administration. We compared the vasoconstrictor responses to ANG II infusions before and during tempol with or without NLA administration, and these responses were calculated by the changes observed due to ANG II infusion from the baseline values just before infusion. In time control experiments (n = 3), ANG II infusions were repeated with a gap period of 1 h without coadministration of tempol. This interval period of 1 h was somewhat similar to the

Table 2. Responses to ANG II before and during tempol in NLA-treated dogs (n = 6)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Enal</th>
<th>Enal + ANG II</th>
<th>Enal + NLA</th>
<th>Enal + NLA + Tempol</th>
<th>Enal + NLA + Tempol + ANG II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic arterial pressure, mmHg</td>
<td>137±6</td>
<td>132±5</td>
<td>134±4</td>
<td>138±7</td>
<td>141±8</td>
<td>138±7</td>
</tr>
<tr>
<td>Renal blood flow, ml/min·1.73 g−1</td>
<td>3.3±0.5</td>
<td>4.0±0.5*</td>
<td>2.9±0.3†</td>
<td>2.6±0.3†</td>
<td>2.0±0.3‡</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td>Renal vascular resistance, mmHg·ml·min·1.73 g−1</td>
<td>45.4±3.2</td>
<td>34.6±2.4*</td>
<td>48.4±4.6†</td>
<td>55.9±9.9†</td>
<td>76.5±21‡</td>
<td>56.8±2.9</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml/min·1.73 g−1</td>
<td>0.78±0.06</td>
<td>0.83±0.1</td>
<td>0.80±0.07</td>
<td>0.75±0.10</td>
<td>0.53±0.07†</td>
<td>0.75±0.07</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.37±0.07</td>
<td>0.35±0.06</td>
<td>0.48±0.05†</td>
<td>0.50±0.07</td>
<td>0.49±0.10</td>
<td>0.54±0.05</td>
</tr>
<tr>
<td>Urine flow, μl/min·1.73 g−1</td>
<td>6.7±2.0</td>
<td>10.0±1.7*</td>
<td>5.4±0.7†</td>
<td>4.2±0.4‡</td>
<td>3.1±0.4</td>
<td>9.1±4.6</td>
</tr>
<tr>
<td>Sodium excretion, μmol/min·1.73 g−1</td>
<td>1.9±0.8</td>
<td>2.3±0.5*</td>
<td>1.1±0.2†</td>
<td>0.8±0.1†</td>
<td>0.6±0.1</td>
<td>1.4±0.3</td>
</tr>
<tr>
<td>Fractional excretion of sodium, %</td>
<td>1.4±0.5</td>
<td>1.6±0.2*</td>
<td>1.1±0.1†</td>
<td>0.7±0.1†</td>
<td>0.7±0.1</td>
<td>1.2±0.3</td>
</tr>
<tr>
<td>Potassium excretion, μmol/min·1.73 g−1</td>
<td>0.37±0.03</td>
<td>0.45±0.07</td>
<td>0.34±0.03</td>
<td>0.35±0.04</td>
<td>0.26±0.04</td>
<td>0.39±0.07</td>
</tr>
<tr>
<td>8-Isoprostane excretion, pg·min·1.73 g−1</td>
<td>49.8±10.0</td>
<td>30.6±4.7*</td>
<td>49.2±10.9†</td>
<td>40.9±3.5§</td>
<td>31.4±5.0</td>
<td>29.3±8.6</td>
</tr>
<tr>
<td>H2O2 excretion, μmol/min·1.73 g−1</td>
<td>1.98±0.92</td>
<td>1.51±0.42</td>
<td>1.71±1.0</td>
<td>2.45±1.3</td>
<td>1.42±0.74</td>
<td>1.05±0.7</td>
</tr>
<tr>
<td>Nitrite/nitrate excretion, mmol·min·1.73 g−1</td>
<td>0.9±0.33</td>
<td>1.23±0.41</td>
<td>0.45±0.15†</td>
<td>0.37±0.17†</td>
<td>0.41±0.13</td>
<td>0.52±0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 in each parameter except H2O2 excretion in which n = 5. NLA, nitro-l-arginine. P < 0.05 vs. control value (*), vs. Enal values (†), and vs. Enal + NLA values (‡). §, 0.1 > P > 0.05 vs. Enal period.
usually lapsed time between two infusions of ANG II before and during tempol administration. It was observed that renal responses to ANG II during the first administration (RBF −15.0 ± 4.0%, RVR +23.1 ± 2.5%, and UNaV −43.4 ± 7.9%) were not different from the responses during the second administration (RBF −15.1 ± 3.2%, RVR +23.4 ± 3.4%, and UNaV −49.5 ± 2.6%). It is to be noted here that our previous studies in dogs (19–21) demonstrated the maximal effects of tempol within 30 min of infusion that remained unchanged for at least 2 h. Two infusions of ANG II before tempol administration in NOS-inhibited dogs were also made within 2 h of NLA administration. During long-term infusion of tempol in salt-depleted anesthetized dogs, we have also observed that renal responses to tempol reached their maximum within 10–15 min of infusion and then remain unaltered during >60 min of the infusion period (unpublished observation). Thus the attenuation of renal responses to ANG II observed in the tempol-treated dogs before and during NOS inhibition was unlikely to be contributed by the time-dependent changes in these experiments.

DISCUSSION

In the present investigation, we have demonstrated that RBF and excretory responses to ANG II infusion are attenuated during intra-arterial infusion of a SOD mimetic, tempol, both in intact and in NOS-inhibited dogs. The marked reduction in GFR in response to ANG II, observed in NOS-inhibited dogs, was also considerably attenuated during tempol administration. As a SOD mimetic agent, tempol effectively scavenges and chemically reduces the O$_2^·$ radicals (23). Thus the findings in the present investigation clearly indicate that O$_2^·$ generation is partly involved in mediating renal responses to ANG II. Scavenging of O$_2^·$ by tempol can attenuate the vasoconstrictor response to ANG II either by increasing NO bioavailability (5, 28, 39) and/or by reducing its direct vascular effects on intracellular calcium (34, 35). Because tempol attenuates ANG II-induced vasoconstriction in NOS-inhibited dogs, it is conceivable that ANG II-induced O$_2^·$ generation also exerts its direct effects on the renal vasculature. Such direct effects of O$_2^·$ were also evident in our earlier study (20) that showed an enhancement of the renal vasoconstrictor response to SOD inhibition in NOS-inhibited dogs. However, the possibility of an incomplete blockade of NO formation in the renal vessels with the dose of NLA used in the present study could not be ruled out, since we have not made any direct measurement of renal content of NO in these experiments. However, functional evidence from our initial experiments (19) indicated that this dose of NLA exerted a maximal renal vasoconstrictor response, indicating near complete blockade of NO formation in the kidney. It may also be argued that a change in intrarenal H$_2$O$_2$ rather than O$_2^·$ level because of tempol infusion (4) might have influenced these renal responses to ANG II. Although we have not made any direct measurement of the changes in intrarenal O$_2^·$ or H$_2$O$_2$ concentrations in the present study, this possibility seems unlikely, since H$_2$O$_2$ was shown to exert vasoconstrictor effects in the kidney (22) that should
enhance, not attenuate, the renal actions of ANG II. Although tempol infusion was shown to cause a mild increase in H2O2 level in the rat renal tissue (4), the present experiments in dogs demonstrate that the urinary excretion rate of H2O2 was not increased by tempol infusion. It was also reported earlier that tempol administration did not alter the tissue H2O2 level in vitro (10).

The involvement of O2− in the renal vascular responses to ANG II demonstrated in the present study are in agreement with the results of another study conducted in our laboratory (11) in which the renal vasoconstrictor response to ANG II administration was shown to be comparatively less in mice lacking the gp91phox subunit of NADPH oxidase (an enzyme necessary for ANG II-induced O2− production) than in wild-type mice. Renal vasoconstrictor response to ANG II infusion in rats was also attenuated during tempol infusion and by the NADPH oxidase inhibitor apocynin (18). In humans, forearm blood flow responses to ANG II injection were attenuated during vitamin C administration (5). Collectively, these findings indicate that the vasoconstrictor action of ANG II is partly mediated by the generation of O2−. It was shown in an in vitro study in isolated rat kidney (8) that ANG II administration facilitates the autoregulatory response through increased O2− formation mediated via ANG II type 1 receptors. Although previous studies in dogs provided no evidence for significant involvement of NO in the maintenance of normal autoregulatory behavior of RBF (19), the results from some recent in vivo studies, particularly in rats, showed that acute inhibition of NOS resulted in autoregulatory resetting of RBF (14) and also showed improvement of impaired autoregulation in some models of hypertensive rats (36, 38). Considering these facts, it is conceivable that such improvement of autoregulatory behavior during NOS inhibition could be the result of possible enhancement of O2− activity (8, 21) and thus suggest an important interactive role of O2− and NO in the control of renal function during an elevated condition of the renin-angiotensin system. It may also be argued that variations in prostaglandin synthesis may be involved in these responses to ANG II during NLA or tempol administration, since an interaction between cyclooxygenase and the renin-angiotensin-aldosterone system is also known to play a role in the regulation of kidney function (28). In the present study, we have not measured any possible changes in intrarenal prostaglandin synthesis in response to ANG II infusion. However, we have plans to examine these interactions further in our future studies using inhibitors of prostaglandin synthesis.

Similar to the findings in a study conducted earlier in conscious dogs (1), the present study showed that ANG II caused a marked decrease in GFR during NOS inhibition. However, we have further observed that this ANG II-induced marked reduction in GFR in NLA-treated dogs was significantly attenuated during tempol infusion (Fig. 6). The slight decrease in GFR resulting from ANG II in intact dogs was also abolished during tempol administration (Fig. 2). These results show that O2− production contributes to the GFR responses to ANG II. Previous studies (8, 11, 39) also supported an inter-
action between NO and O$_2^-$ in the regulation of GFR. In isolated rat kidney preparations, scavenging of O$_2^-$ by tempol completely prevented facilitation of the tubuloglomerular feedback (TGF) response by ANG II (8). Welch et al. (39) demonstrated that the reduction in TGF in response to microperfusion of a NO donor compound in the macula densa lumen was less in SHR than in normotensive Wister-Kyoto rats. The difference in the responses was abolished by confusion with tempol, suggesting an interaction between O$_2^-$ and NO in regulating TGF-mediated changes in afferent arteriolar tone in SHR, a model known to have enhanced oxidative stress (30).

We have also reported (11) that ANG II administration caused no change in GFR in wild-type mice but caused an increase in GFR in knock-out (KO) mice lacking the gp91phox subunit of NAD(P)H oxidase. This finding in our mice study (11) indicates that a lack of O$_2^-$ production in response to ANG II in KO mice exerted proportionately less constrictor action on the afferent than on the efferent arterioles, resulting in an increase in glomerular pressure, thus leading to an increase in GFR. The results in the investigation also suggest that vasoconstrictor action of O$_2^-$ in the afferent arteriole during ANG II infusion was mostly counteracted by NO in the intact state but unopposed in the state of NOS inhibition, thus causing a marked reduction in GFR. It is interesting to note that tempol treatment in both intact and NO-blocked dogs caused improvement in GFR, indicating that O$_2^-$ may predominantly affect the preglomerular vascular resistance (8, 11, 39). However, our present in vivo experiments do not allow us to comment on the relative contribution of afferent and efferent arteriolar resistances affected by O$_2^-$ formation. Moreover, a prediction of alterations in segmental vascular resistance based on the changes in filtration fraction was also seriously questioned in a previous study using a computer-simulated experimental model of combined afferent and efferent resistance changes (3).

As observed in our earlier study (21), tempol administration in the present study did not cause any appreciable changes in RBF, V, and UNaV before NOS inhibition (Tables 1 and 2). However, tempol infusion during NLA administration resulted in increases in V and UNaV (Table 2) without appreciable changes in renal hemodynamics, as previously reported (21). In the condition of NOS inhibition, tempol administration is not expected to cause an increase in RBF, since the vasodilator effect of scavenging O$_2^-$ is primarily the result of the action of increased NO bioavailability in the vessel (21). The present findings further support our earlier contention (21) that the basal O$_2^-$ activity remained at a minimal level in normal conditions because of antioxidative effects of endogenous NO. During tempol administration, ANG II-induced reduction in V and in UNaV was attenuated in both intact and NOS-inhibited dogs. There were no changes in FE$_{Na^+}$ in response to ANG II during tempol administration in NLA-treated dogs. Such marked attenuation of the renal excretory responses to ANG II during tempol infusion provides evidence in support of the involvement of O$_2^-$ in ANG II-induced changes in tubular reabsorptive function. Results in NOS-inhibited dogs strongly suggest that ANG II-induced O$_2^-$ production can affect tubular reabsorptive function independent of its effects on NO metabolism. These findings suggest that the renal excretory responses to ANG II are, at least in part, attributed to the tubular effects of O$_2^-$ that are aggravated in the condition of NO deficiency. These results are in agreement with the reported in vitro study (26) in which O$_2^-$ was shown to enhance renal tubular sodium transport in the thick ascending limb of the loop of Henle.

In these experiments in dogs, we have observed that ANG II infusion increased U$_{ISOV}$ and decreased U$_{NOXV}$ (Tables 1 and 2), as reported in other studies in rats (18). These effects of ANG II on U$_{ISOV}$ and U$_{NOXV}$ were abolished during tempol and NLA infusion (Tables 1 and 2). Although an increase in U$_{ISOV}$ during ANG II is expected, the reason for a decrease in U$_{NOXV}$ in response to ANG II is not yet clear. However, although no direct evidence is currently available, one explanation could be that increases in O$_2^-$ production during ANG II administration interacted with NO, and this chemical reaction between O$_2^-$ and NO could reduce the formation of nitrite/nitrates. As expected, U$_{NOXV}$ was decreased significantly during NLA infusion in these enalaprilat-treated dogs compared with values observed during enalaprilat infusion alone. U$_{ISOV}$ level was also higher during NLA infusion compared with values with enalaprilat alone, further supporting our earlier observation (21) that O$_2^-$ levels in the kidney are enhanced during NOS inhibition. It has been observed that ACE inhibition decreases U$_{ISOV}$ and increases U$_{NOXV}$ (Tables 1 and 2). These findings are expected, since ACE inhibition decreased endogenous formation of ANG II that could cause a decrease in endogenous formation of O$_2^-$ radical that is reflected in U$_{ISOV}$. Reduced O$_2^-$ formation thus leads to increases in endogenous NO bioavailability that have been reflected in increases in U$_{NOXV}$.

Our study was not designed to define the source of O$_2^-$ generation in the kidney during ANG II infusion. However, ANG II has been shown to stimulate O$_2^-$ generation in the vascular smooth muscle (27) and mesangial cells (12). The key enzyme systems involved in the ANG II-induced production of O$_2^-$ are considered to be NADH and NADPH oxidase enzymes (27). It has also been demonstrated that the thick ascending limbs of the loop of Henle are the major site for NADH oxidase enzyme, which could enhance production of O$_2^-$ due to the action of ANG II (16). Thus it is possible that O$_2^-$ generation via NAD(P)H oxidase enzyme activation is involved in mediating renal actions of acute ANG II infusion in the present study.

The findings in the present investigation indicate that ANG II caused an enhanced production of O$_2^-$ that alters renal hemodynamics and enhances tubular retention of salt and water, which could be involved in the development of hypertension during chronic administration of a subpressor dose of ANG II (28). It was reported that chronic infusion of low doses of ANG II is accompanied by the stimulation of oxidative stress, as measured by a significant increase in plasma 8-isoprostanol levels (9). Although oxidative stress has been proposed to play a role in the development of ANG II-dependent hypertension, the exact mechanism involved in this pathophysiology is not yet clearly defined. The findings in the present study emphasize that the elucidation of complete interactions between ANG II, O$_2^-$, and NO is essential to further understand the renal mechanisms that are involved in the pathophysiology of ANG II-dependent hypertension.

In conclusion, the results of the present investigation demonstrate that the renal responses to ANG II are partly mediated by the generation of O$_2^-$ and its interaction with NO. These findings further indicate that, in the condition of NO defi-
iciency, O₂ greatly influences ANG II-induced sodium reabsorptive function in the kidney.

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REFERENCES


