Role of Extracellular Superoxide Dismutase in the Mouse Angiotensin Slow Pressor Response

William J. Welch, Tinatin Chabrashvili, Glenn Solis, Yifan Chen, Pritmohinder S. Gill, Shakil Aslam, Xiaoyan Wang, Hong Ji, Kathryn Sandberg, Pedro Jose, Christopher S. Wilcox

Abstract—Low rates of angiotensin II (Ang II) infusion raise blood pressure, renal vascular resistance (RVR), NADPH oxidase activity, and superoxide. We tested the hypothesis that these effects are ameliorated by extracellular superoxide dismutase (EC-SOD). EC-SOD knockout (−/−) and wild type (+/+) mice were equipped with blood pressure telemeters and infused subcutaneously with Ang II (400 ng/kg per minute) or vehicle for 2 weeks. During vehicle infusion, EC-SOD −/− mice had significantly (P<0.05) higher MAP (+/+: 107±3 mm Hg versus −/−: 114±2 mm Hg; n=11 to 14), RVR, lipid peroxidation, renal cortical p22phox expression, and NADPH oxidase activity. Ang II infusion in EC-SOD +/+ mice significantly (P<0.05) increased MAP, RVR, p22phox, NADPH oxidase activity, and lipid peroxidation. Ang II reduced SOD activity in plasma, aorta, and kidney accompanied by reduced renal EC-SOD expression. During Ang II infusion, both groups had similar values for MAP (+/+ Ang II: 125±3 versus −/−: Ang II: 124±3 mm Hg; P value not significant), RVR, NADPH oxidase activity, and lipid peroxidation. SOD activity in the kidneys of Ang II–infused mice was paradoxically higher in EC-SOD −/− mice (+/+: 8.8±1.2 U/mg protein−1 versus −/−: 13.7±1.6 U/mg protein−1; P<0.05) accompanied by a significant upregulation of mRNA and protein for Cu/Zn-SOD. In conclusion, EC-SOD protects normal mice against oxidative stress by attenuating renal p22 phox expression, NADPH oxidase activation, and the accompanying renal vasoconstriction and hypertension. However, during an Ang II slow pressor response, renal EC-SOD expression is reduced and, in its absence, renal Cu/Zn-SOD is upregulated and may prevent excessive Ang II–induced renal oxidative stress, renal vasoconstriction, and hypertension. (Hypertension. 2006;48:934-941.)

Key Words: oxidative stress • hypertension • renal • kidney • renal circulation • nitric oxide • endothelium

An increase in reactive oxygen species (ROS) in the blood vessels and kidneys is reported in several experimental animal models of hypertension and in human essential and renovascular hypertension.1 Infusions of angiotensin II (Ang II) increase blood pressure (BP), markers of oxidative stress, and renal expression of the p22phox and Nox-1 components of renal NADPH oxidase.2 These effects seem specific for Ang II, because similar pressor infusions of norepinephrine into rats do not induce oxidative stress in blood vessels.3 An increased production of superoxide (O2•−) reduces bioactive NO4 and contributes to vascular and renal injury in chronic hypertension.5,6 O2•− dismutase (SOD) metabolizes O2•− to H2O2, which is further metabolized to inactive products by peroxidases. Hypertension can be moderated or prevented by gene transfer of extracellular (EC)-SOD7 or by administration of tempol,8 which is a nitroxide SOD mimic.

The 3 isoforms of SOD are localized to the kidney.9 EC-SOD is located on cell membranes of endothelial cells and vascular smooth muscle cells.10 EC-SOD −/− mice have endothelial dysfunction in conduit blood vessels that is ascribed to an impaired NO bioavailability.11 EC-SOD expression in blood vessels is increased during pressor infusions of Ang II and may thereby limit the increase in vascular O2•−.12 This may explain the finding that EC-SOD −/− mice have an exaggerated early increase in BP during pressor infusions of Ang II.13 Ang II infusions at initially suppressor rates leads to a slow development of hypertension and renal vasoconstriction that depends on O2•−, because these effects are prevented by tempol.13 This slow pressor response likely entails a renal mechanism, because the hypertension depends on salt intake14 and is accompanied by a preferential renal vasoconstriction,15 enhanced renal afferent arteriolar constrictor response to Ang II,16 and salt retention.17 A slow pressor response is seen in mice,13 rats,18,19 dogs,20 rabbits,21 and humans.22 It has been considered a model of human hypertension, because it is accompanied by only modest elevations in plasma Ang II concentrations.19,23 The role of EC-SOD in the kidney in this model is quite unclear, because renal cortical EC-SOD expression in the rat is downregulated by a 2-week infusion of Ang II at a slow pressor rate.2 Therefore,
Infusion of Ang II at a slow pressor rate of 400 ng/kg for 6 days before and during subcutaneous infusion of Ang II at a slow pressor rate of 400 ng/kg/min (top) or a pressor rate of 1000 ng/kg/min (bottom) from day 1. Compared with EC-SOD +/+; *P<0.05; **P<0.01.

Methods

Animal Preparation
The protocol was approved by the Georgetown University Animal Care and Use Committee. Young adult male EC-SOD +/+ and −/− mice were bred from +/+ founders kindly provided by Dr Marklund (Umea University, Umea, Sweden). They were developed in a C57B6 mouse background and reproduced and developed normally as described previously. For the first series, mice were instrumented with indwelling radiotelemeters (DSI) connected to a catheter in a carotid artery and placed within the abdomen 2 weeks before placement of osmotic minipumps as described previously.13 Mice were anesthetized with isoflurane (1.0 to 1.5% in 100% O₂) before insertion of telemeters and allowed to recover from the surgery for 12 days before the start of BP recording. Basal recordings of MAP and heart rate (HR) were measured continuously by telemetry for 3 days. Thereafter, mice were anesthetized with isoflurane for insertion of osmotic minipumps (Direct Corp) to deliver Ang II (400 ng kg⁻¹ min⁻¹) or vehicle (Veh) subcutaneously for 2 weeks. An additional group received a higher pressor rate of Ang II infusion (1000 ng kg⁻¹ min⁻¹, SC). The mean values for 24-hour periods are reported. For subsequent series, mice were prepared without placement of telemeters, and Ang II was infused only at the slow pressor rate.

Urine Collection
At day 12 of infusion, mice were placed in metabolic cages (Hatteras Instruments). A 24-hour urine sample was collected in the presence of antibiotics (penicillin G: 0.8 mg; streptomycin: 2.6 mg; and amphotericin B: 5 mg) for excretion of 8-isoprostaglandin F₂α and malondialdehyde (MDA), as described.13,25

Renal Function
On day 13 of infusion, mice in series 2 were anesthetized with thiobarbitual (Inactin, 50 mg kg⁻¹) and ketamine (40 mg kg⁻¹). Cannulae were placed in the jugular vein (for infusion of fluids and renal function markers), the femoral artery (for direct measurement of MAP and HR), and the bladder (for the collection of urine). A tracheostomy was performed to permit free, uninterrupted respiration of room air. [³H]-Inulin was infused at 0.1 μCi·min⁻¹·kg⁻¹ for the measurement of glomerular filtration rate. [³H]-Para-amo-nipper (PAH) was infused at 0.2 μCi·min⁻¹·kg⁻¹. Blood was collected from the femoral artery and renal vein at the end of the collection period to measure hematocrit and renal PAH extraction. Renal plasma flow (RPF) was calculated from the clearance of PAH factored by its renal extraction. Renal blood flow was calculated from RPF factored by 1–hematocrit. Renal vascular resistance (RVR) was calculated from MAP factored by RBF. In separate groups, plasma was obtained, and the aorta and kidneys were harvested and prepared for further analysis.

Biochemical Assays
NO−₃+NO−₂ (NOx) was measured in an NO chemiluminescence analyzer (model 270B, Stevers Instruments). 8-Isoprostaglandin F₂α was measured using an HPLC assay.

MAP (mm Hg) of Conscious and Anesthetized Mice: Effects of Strain and Ang II Infusion

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of Mice</th>
<th>Rate of Ang II Infusion, ng/kg⁻¹·min⁻¹</th>
<th>MAP Before, mm Hg</th>
<th>MAP on Day 12 of Ang II, mm Hg</th>
<th>Change in MAP With Ang II, mm Hg</th>
<th>Effects of Ang II, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conscious mice, telemetry</td>
<td></td>
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<tr>
<td>EC-SOD (+/+</td>
<td>8</td>
<td>400</td>
<td>108.7±3.8</td>
<td>134.6±3.3</td>
<td>24.4±3.45</td>
<td>P&lt;0.001</td>
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<tr>
<td>P (+/+ vs −/−)</td>
<td></td>
<td></td>
<td>P&lt;0.05</td>
<td>ns</td>
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<tr>
<td>Anesthetized mice</td>
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</tr>
<tr>
<td>EC-SOD (+/+</td>
<td>11</td>
<td>400</td>
<td>79.6±1.9</td>
<td>92.1±3.0</td>
<td>P&lt;0.001</td>
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<td>P (+/+ vs −/−)</td>
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<td>P&lt;0.05</td>
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<td>Conscious mice, telemetry</td>
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<tr>
<td>EC-SOD (+/+</td>
<td>6</td>
<td>1000</td>
<td>109.8±3.1</td>
<td>149.1±4.3</td>
<td>35.7±6.5</td>
<td>P&lt;0.001</td>
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<td>P (+/+ vs −/−)</td>
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<td>P&lt;0.05</td>
<td>ns</td>
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<tr>
<td>Mean±SEM values. Before indicates mean data over 6 days before insertion of Ang II minipump.</td>
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was measured by enzyme-linked immunoassay (Cayman, Inc) using a method described previously and validated against gas chromatography mass spectrometry. MDA was measured from thiobarbituric acid reactive substances (Zepto Metric Inc) as described previously. NADPH oxidase activity was assessed by measuring O$_2^-$ generation in renal cortex homogenates by lucigenin (5 nM)-enhanced chemiluminescence measured in a luminometer (Porter, Inc, Berthold Autolumat, Berthold Technologies) after the addition of 100 μM of NADPH, as described previously. Plasma renin activity was measured by radioimmunoassay (Disorin). Plasma renin concentration was measured after the addition of supramaximal concentration (0.5 mg/mL) of mouse angiotensinogen (Peninsula).

**Expression of mRNA and Protein for SOD Isoforms p22$^{phox}$ and p47$^{phox}$ in Kidney Cortex**

The expression of these genes was assessed in homogenates of renal cortex using real-time PCR and Western analysis, as described in detail previously.

**SOD Activity**

This activity was evaluated using a modified chemiluminescence technique from the inhibition of O$_2^-$ signals by mouse plasma or homogenates of abdominal aorta or kidney cortex after the addition of xanthine (100 μM) and xanthine oxidase (Sigma-Aldrich). The aorta or kidney cortex was dissected in ice-cold PBS and homogenized at 3000 rpm for 30 minutes. The SOD activity of the supernatant was calculated from a standard curve of inhibition of O$_2^-$ generation by Cu/Zn-SOD (Sigma-Aldrich).

**Statistics**

The differences between EC-SOD $+/+$ and $+/-$ mice, the effects of Ang II versus Veh infusion, and the interaction (effects of strain on the responses to Ang II) were assessed by 2-way ANOVA. When appropriate, a post hoc Dunnett’s t test was applied to detect significant differences between groups. Data are presented as mean±SEM values. Significance is accepted at P<0.05.

**Results**

**MAP of Conscious Mice**

The MAP averaged over 6 control days before infusion was higher in EC-SOD $+/-$ mice ($+/+$: 101±2, n=14 mm Hg, versus $+/-$: 110±3, n=11 mm Hg; P<0.05). The MAP of both strains increased progressively during the first week of Ang II infusion at a slow pressor rate and remained elevated throughout the infusion (Figure 1). The increase in MAP with Ang II infused at a slow pressor rate was similar in EC-SOD $+/+$ and $+/-$ mice (Table). The MAP of mice infused with Veh did not change during the infusion (data not shown). The HR was not affected by strain or infusion of Ang II (data not shown). Mice infused with Ang II at a pressor rate of 1000 ng/kg·min$^{-1}$ had an abrupt increase in MAP, which was greater in EC-SOD $+/-$ than $+/+$ mice on days 2 to 6 of the infusion (Figure 1), confirming a previous study. However, during the second week of infusion of Ang II at the pressor rate, the MAP values became similar in EC-SOD $+/+$ and $+/-$ mice. The EC-SOD $+/-$ mice of this group had a similar increase in MAP over 10 to 13 days of Ang II infusion (Table). Subsequent data refer to Ang II infusion at a slow pressor rate, because the object of this study was to assess the role of EC-SOD in the slow pressor response.

**MAP Under Anesthesia**

The MAP of anesthetized, Veh-infused mice also was higher in EC-SOD $+/-$ than in $+/+$ mice (Table). By day 13 of Ang II infusion at a slow pressor rate, the MAP under anesthesia had increased to a similar value in both strains (Table).

**Renal Function**

The glomerular filtration rate and RPF during Veh infusion were not significantly different between strains and were not significantly altered by Ang II (Figure 2). The RVR during infusion of Veh was higher in EC-SOD $+/-$ mice. During infusion of Ang II, RVR did not increase significantly in EC-SOD $+/-$ mice and became comparable to values in EC-SOD $+/+$ mice (Figure 2).

**Plasma Renin**

The plasma renin activity and plasma renin concentration during infusion of Veh did not differ between strains (data not shown). These parameters were not measured in Ang II-infused mice.

**Figure 2.** Mean±SEM values for glomerular filtration rate, RPF, and RVR in EC-SOD $+/+$ and $+/-$ mice on day 12 or 13 of infusion of Veh or Ang II. Compared with Veh in same strain: **P<0.01. Compared with EC-SOD $+/+$, same treatment group: β indicates P<0.01.

**Figure 3.** Mean±SEM values for NOx excretion in EC-SOD $+/+$ and $+/-$ mice on day 12 or 13 after infusion of Veh or Ang II. Compared with Veh in same strain: **P<0.01. Compared with EC-SOD $+/+$, same treatment group: β indicates P<0.01.
Markers of NO and Oxidative Stress
During Veh infusion, EC-SOD −/− mice had a reduced excretion of NOx, but, during Ang II, this increased only in EC-SOD −/− mice (Figure 3). EC-SOD −/− mice had an increased excretion of 8-isoprostaglandin (+/+: 1.3±0.3; n=6 versus −/−: 2.1±0.2; n=6; pg 24 h−1; P<0.01) and MDA (+/+: 31±3; n=6 versus −/−: 59±6; n=6; nmol 24 h−1; P<0.01; Figure 4). Ang II increased the excretion of both markers significantly (P<0.01) in EC-SOD +/+ mice but did not change the excretion of either marker significantly in −/− mice. This resulted in similar values for NOx, 8-isoprostaglandin, and MDA in EC-SOD −/− and +/+ mice during infusion of Ang II.

Renal NADPH Oxidase Activity and Expression of p22phox and p47phox
The NADPH oxidase activity of renal cortical homogenates was greater in Veh-infused EC-SOD −/− mice (+/+: 7.2±0.9; n=6 versus −/−: 11.2±1.1; n=6; nmol/mg of protein−1; P<0.01; Figure 5). This increased significantly (P<0.001) with Ang II infusion in EC-SOD +/+ mice but not in −/− mice. The renal cortical expression of p22phox protein in EC-SOD −/− mice was greater (P<0.001) than in EC-SOD +/+ mice during Veh infusion but increased with Ang II only in EC-SOD +/+ mice (Figure 6). There were no differences in expression of p47phox (Figure 6).

![Figure 5. Mean±SEM values for NADPH oxidase activity in of the kidney cortex of EC-SOD +/+ and −/− mice on day 12 or 13 of infusion of Veh (●) or Ang II (●) in EC-SOD +/+ and −/− mice. Compared with Veh, in same strain: ***P<0.005. Compared with EC-SOD +/+ , same treatment group: β indicates P<0.01.](image)

Total SOD Activity
SOD activity was not detectable in the plasma from EC-SOD −/− mice. The SOD activity in the plasma, aorta, and kidney cortex of EC-SOD +/+ mice was reduced by Ang II infusion (Figure 7). The SOD activity in the aorta of EC-SOD −/− mice was lower than in EC-SOD +/+ mice during infusion of Veh (+/+: 18.2±3.2; n=6 versus −/−: 13.1±1.2; n=6; U/mg of protein−1; P<0.05; Figure 7) but was not changed significantly by Ang II. The SOD activity in the renal cortex was similar in both strains during Veh infusion (+/+: Veh: 14.9±3.5; n=6 versus −/− Veh: 12.1±1.6; n=6; U/mg of protein−1; P not significant; Figure 7) and was reduced during infusions of Ang II only in EC-SOD in +/+ mice, resulting in a paradoxical higher (P<0.05) SOD activity in the renal cortex of EC-SOD −/− mice compared with +/+ mice during infusion of Ang II (Figure 7).

Expression of SOD Isoforms in Kidney Cortex
As anticipated, the mRNA expression for EC-SOD in the kidneys of −/− mice was not detectable. The mRNA and protein expression for EC-SOD was reduced (P<0.05) by Ang II in EC-SOD +/+ mice, confirming our previous results in normal rats (Figure 8). The mRNA and protein expression for Mn-SOD in the kidney cortex was unchanged by Ang II in either strain (Figure 9). The expression of IC-SOD or Cu-Zn-SOD protein but not mRNA in the kidney cortex of Veh-infused mice was reduced in EC-SOD −/− mice. During Ang II, the mRNA and protein expression for IC-SOD increased in EC-SOD −/− but not in EC-SOD +/+ mice, and the protein concentration became higher in the kidney cortex of the EC-SOD −/− strain (Figure 9).

Discussion
The main new findings of this study are that the MAP (whether measured in conscious mice by telemetry or in anesthetized mice by direct arterial cannulation) was modestly, but significantly, higher in EC-SOD −/− mice. This was accompanied by an increase in RVR and evidence of increased oxidative stress (increased 8-isoprostaglandin, PGF2α, and MDA excretion) and decreased NO generation (decreased NOx excretion). The increased oxidative stress in EC-SOD −/− mice was accompanied by reduced SOD activity in both the plasma and aorta and increased p22phox expression and NADPH oxidase activity in the kidney. These data indicate that the oxidative stress of EC-SOD −/− mice

Figure 4. Mean±SEM values for renal excretion of 8-isoprostaglandin F2α, and MDA in EC-SOD +/+ and −/− mice on day 12 or 13 of infusion of Veh (●) or Ang II (●). Compared with Veh in same strain: ***P<0.005. Compared with EC-SOD +/+ , same treatment group: β indicates P<0.01.
may originate from both a decreased metabolism of $\text{O}_2^-$ by EC-SOD and an increased generation of $\text{O}_2^-$ by NADPH oxidase. The reduced SOD activity in the aorta of EC-SOD $\text{+/+}$ mice supports previous studies suggesting that EC-SOD is a major defense system against oxidative stress in blood vessels.\textsuperscript{5,11,28} In contrast, the maintained SOD activity in the kidney cortex of SOD $\text{+/+}$ mice indicates that other SOD isoforms contribute to oxidative defense in the kidney.

We found that Ang II infused at a pressor rate increased the MAP more rapidly in EC-SOD $\text{+/+}$ than $\text{+/-}$ mice similar to previous findings.\textsuperscript{11} In contrast, Ang II infused at a slow pressor rate led to similar increases in MAP in EC-SOD $\text{+/+}$ and $\text{-/-}$ mice. The increase in excretion of lipid peroxidation products produced by the slow pressor Ang II infusion in normal mice were unexpectedly absent in EC-SOD $\text{-/-}$ mice. This may relate to the unchanged SOD activity in the aorta and kidney and the unchanged renal cortical expression of p22\textsuperscript{phox} and NADPH oxidase activity with Ang II in EC-SOD $\text{-/-}$ mice. In contrast, the SOD activity in the plasma, aorta, and kidneys of EC-SOD $\text{+/+}$ was reduced, and the p22\textsuperscript{phox} expression and NADPH oxidase activity of the kidney cortex was increased during Ang II infusion at a slow pressor rate. The reduced SOD activity in the aorta and kidney cortex of EC-SOD $\text{+/+}$ mice infused with Ang II at a slow pressor rate was accompanied by a reduced mRNA and protein expression for EC-SOD, confirming previous studies in normal rats.\textsuperscript{2} In contrast, the SOD activity was maintained in the aorta and actually was increased in the kidney cortex during infusion of Ang-II into EC-SOD $\text{-/-}$ mice. This may be explained by the increased expression of the mRNA and protein for IC-SOD in the kidneys of EC-SOD $\text{-/-}$ mice infused with Ang II.

These data suggest that 3 factors could account for the failure of Ang II infusion to increase ROS in EC-SOD $\text{-/-}$ mice. First, Ang II infusion at a slow pressor rate reduces the expression of EC-SOD in the wild-type kidney (Figure 8). Thus, this down-regulation of EC-SOD could minimize the differences in oxidative stress between EC-SOD wild-type and knockout animals during Ang II infusion. This finding that Ang II infusion at a slow pressor rate downregulates the mRNA and protein expression for EC-SOD in the kidneys of wild-type mice (Figure 8) contrasts with the upregulation of...
EC-SOD expression in the aorta of rats infused with Ang-II at a pressor rate.12 Second, in the absence of EC-SOD, Ang II infusion upregulated the mRNA and protein expression of IC-SOD in the kidney cortex (Figure 9). This upregulation of IC-SOD may be sufficient to offset any reduction in SOD activity because of the loss of EC-SOD. Third, in contrast to wild-type mice, Ang II infusion failed to increase p22phox expression (Figure 6) or NADPH oxidase activity (Figure 5) in the kidney cortex of EC-SOD −/− mice. Collectively, these results may explain the absence of Ang II–induced increases in ROS in EC-SOD −/− mice. Our finding of a modest increase in MAP in conscious and anesthetized EC-SOD −/− mice conflicts with the finding of Jung et al11 and Jonsson et al29 of similar systemic BPs in EC-SOD +/+ and −/− mice. The difference from previous studies might relate to the use of tail-cuff BPs in one study11 and the use of older mice in the other.29 EC-SOD is expressed in blood vessels primarily on the surface of vascular smooth muscle cells and the subendothelial space.10,30 It contains a heparin-binding domain that binds to proteoglycans expressed on cell surfaces.31

The finding that SOD activity in Veh-infused EC-SOD −/− mice was undetectable in the plasma and was reduced in the aorta by ≈33% but was unchanged in the kidneys suggests that the increase in oxidative stress observed in EC-SOD −/− mice is primarily because of the loss of EC-SOD in the plasma and partly because of the loss in the aorta, whereas EC-SOD in the kidney makes little, if any, contribution.

Our study has disclosed 2 factors that may contribute to the higher basal levels of MAP in EC-SOD −/− mice. First, the increase in RVR could raise MAP by directly increasing peripheral resistance. The resulting increase in afferent arteriolar resistance could attenuate the ability of pressure/naturenisk mechanisms to compensate for the elevation in BP. This possibility is consistent with observations that narrowed afferent arterioles predict the development of hypertension in the spontaneously hypertensive rat13 and accompany hypertension in humans.33 Second, oxidative stress could contribute to the higher BPs found in EC-SOD −/− mice, because oxidative stress induces salt sensitivity,8,34 increases vascular reactivity,16 and contributes to Ang II–induced hypertension.6,35

Studies of the response of large blood vessels to endothelium-derived relaxation factor/NO demonstrate that EC-SOD exerts major control over vascular O2− and bioavailable NO and thereby modulates the endothelium-derived relaxation factor response.11,31,36 Because IC-SOD or Cu/Zn-SOD accounts for 60% to 80% of SOD activity in the kidney, we assessed whether it might compensate for a deficiency of EC-SOD. The levels of IC-SOD and Mn-SOD protein were unchanged in the aorta of EC-SOD −/− mice,11 but IC-SOD protein was reduced in the kidney cortex of these mice. During infusion of Ang II, the mRNA and protein for IC-SOD were increased in the kidney cortex of EC-SOD −/− mice, which may thereby account for the maintained SOD activity.

An increase in BP, RVR, and reactivity of the afferent arteriole to low-dose Ang II infusion depends on O2−, because these effects can be prevented by the SOD mimetic tempol.13,16,18,21 Ang II infusion increases the expression of p22phox and renal NOX-1 and increases NADPH oxidase activity, 8-isoprostaglandin excretion, and BP. We showed recently that these effects can be prevented by silencing the p22phox gene.15 The increase in renal p22phox expression is itself dependent on O2−, because it can be prevented by coinfusion of tempol.18 Apparently, p22phox expression can function as a feed-forward activator of NADPH oxidase during oxidative stress. This concept is consistent with the present finding that the increased expression of p22phox in the kidneys of EC-SOD −/− mice (Figure 6) is associated with increased markers of oxidative stress (Figure 4). Infusion of Ang II into EC-SOD −/− mice failed to increase p22phox expression or NADPH oxidase activity in the kidneys or the excretion of isoprostanes or MDA but evoked a similar increase in MAP relative to the effects of Ang II in EC-SOD +/+ mice (Table 1). The EC-SOD −/− mice had evidence of oxidative stress and a raised MAP before the start of the Ang II infusion. This suggests that an increase in oxidative stress above an elevated level is not required for the increase in MAP during an Ang II slow pressor response in the mouse. In the present study, the paradoxical increase in SOD activity in the kidneys of EC-SOD −/− mice infused with slow pressor doses of Ang II may have protected these mice from an excessive increase in RVR and thereby limited the increase in MAP. In contrast, the reduced SOD activity in their aorta of EC-SOD −/− mice may explain why BP increases more rapidly in these mice during infusion of Ang II at a pressor rate, because aortic EC-SOD plays a major role in vascular mechanisms of hypertension (see Figure 1 and Reference 11).

Perspectives
The expression of EC-SOD in blood vessels is reduced by NO deficiency,37 transforming growth factor β,38 hypero-
mocysteinemia,39 and in patients with coronary artery disease.40 Some 2% to 5% of the normal population31 and 13% of diabetics with end stage renal disease41 carry a substitution of arginine-213 by glycine, which prevents the binding of EC-SOD to blood vessels and thereby enhances endothelial NO bioinactivation by O2.31 Normal subjects or diabetic patients with end stage renal disease who express this arginine-213 by glycine substitution have almost a doubled risk of ischemic heart disease.41-42 The EC-SOD →−/− mouse is a good model for human subjects with ineffective EC-SOD expression. Our finding that this EC-SOD →−/− mouse has an elevated BP, renal vasoconstriction, oxidative stress, increased p22phox expression, and NADPH oxidase activity in the kidney confirms that EC-SOD can be an important factor underlying cardiovascular disease. Moreover, it suggests a therapeutic role for EC-SOD42 or for SOD mimetics, such as the nitroxide tempol,8 to restore SOD activity in those conditions associated with reduced EC-SOD expression or activity. Although these previous results suggest a major role for EC-SOD in protecting large blood vessels from atherosclerosis or its complications, our data demonstrate only a rather small role for EC-SOD in the physiological regulation of MAP in the mouse but an important interaction with NADPH oxidase.

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Disclosures

None.

References


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