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Biology of Free Radicals

Angiotensin-induced defects in renal oxygenation: role of oxidative stress

William J. Welch, Jonathan Blau, Hui Xie, Tina Chabrashvili, and Christopher S. Wilcox
Division of Nephrology and Hypertension and Cardiovascular Kidney Institute, Georgetown University, Washington, DC

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Welch, William J., Jonathan Blau, Hui Xie, Tina Chabrashvili, and Christopher S. Wilcox. Angiotensin-induced defects in renal oxygenation: role of oxidative stress. Am J Physiol Heart Circ Physiol 288: H22–H28, 2005; doi:10.1152/ajpheart.00626.2004.—We tested the hypothesis that superoxide anion (O_2^-) generated in the kidney by prolonged angiotensin II (ANG II) reduces renal cortical PO_2 and the use of O_2 for tubular sodium transport (T_{Na,O_2}). Groups (n = 8–11) of rats received angiotensin II (ANG II, 200 ng·kg^{-1}·min^{-1}·sc) or vehicle for 2 wk with concurrent infusions of a permeant nitroxide SOD mimetic 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (Tempol, 200 nmol·kg^{-1}·min^{-1}) or vehicle. Rats were studied under anesthesia with measurements of renal oxygen usage and P_{O_2} in the cortex and tubules with a glass electrode. Compared with vehicle, ANG II increased mean arterial pressure (107 ± 4 vs. 146 ± 6 mmHg; P < 0.001), renal vascular resistance (42 ± 3 vs. 65 ± 7 mmHg·ml^{-1}·min^{-1}·100 g^{-1}; P < 0.001), renal cortical NADPH oxidase activity (2.3 ± 0.2 vs. 3.6 ± 0.4 nmol O_2^-·min^{-1}·mg^{-1} protein; P < 0.05), mRNA and protein expression for p22^phox (2.1- and 1.8-fold respectively; P < 0.05) and reduced the mRNA for extracellular (EC)-SOD (~1.8 fold; P < 0.05). ANG II reduced the P_{O_2} in the proximal tubule (39 ± 1 vs. 34 ± 2 mmHg; P < 0.05) and throughout the cortex and reduced the T_{Na,O_2} (17 ± 1 vs. 9 ± 2 μmol·μmol^{-1}·min^{-1}; P < 0.001). Tempol blunted or prevented all these effects of ANG II. The effects of prolonged ANG II to cause hypertension, renal vasoconstriction, renal cortical hypoxia, and reduced efficiency of O_2 usage for Na^+ transport accompanied by oxidative stress and increased expression of the p22^phox component of NADPH oxidase and decreased expression of extracellular superoxide dismutase (EC-SOD) (7, 46, 59).

METHODS

Studies were approved by the Georgetown University Animal Care and Use Committee. Adult Wistar-Kyoto (WKY) rats (175–250 g) were supplied by Harlen Sprague Dawley (Madison, WI). They were maintained on a standard rat chow (Purina; St. Louis, MO; Na^+ content 0.3 g/100 g) with free access to food and water until the day of the study.

Protocols. Four groups (n = 8–11) were studied after 12–13 days of subcutaneous infusions from two osmotic minipumps (Alzet). Group 1 received vehicle (Veh; 0.154 M NaCl; 0.2 ml) from each minipump. Group 2 received ANG II (200 ng·kg^{-1}·min^{-1}, Sigma) plus vehicle. This “slow pressor” dose of ANG II produces reproducible hypertension over 2 wk accompanied by oxidative stress and increased renal expression of the p22^phox component of NADPH oxidase and decreased expression of extracellular superoxide dismutase (EC-SOD) (7, 46, 59).

Group 3 received Tempol (200 nmol·kg^{-1}·min^{-1}) plus a vehicle. This dose of Tempol prevents oxidative stress in the SHR (51) and 2K,1C Goldblatt models (62).

Group 4 received ANG II and Tempol.

Animal preparation and clearance measurements. Rats were prepared for clearance and micropuncture as described (60, 64). In brief, animals were anesthetized with thiobarbital (Inactin, 100 mg/kg ip; Research Biochemicals, Natick, MA). A catheter was placed in a jugular vein for fluid infusion and in a femoral artery for recording of mean arterial pressure (MAP). The animals breathed spontaneously via a tracheostomy. A catheter was placed into the bladder to collect urine. The left kidney was exposed by a flank incision, cleaned of connective tissue, and stabilized in a Lucite cup. It was bathed in 0.154 M NaCl equilibrated with air at 37°C. After surgery was completed, rats were infused with a solution of 0.154 M NaCl and 1% albumin at 1.5 ml/h to maintain euvoolemia (64). Studies were begun after 60 min.

For clearance, [3H]inulin (0.1 μCi/ml; ICN Biochemicals, Costa Mesa, CA) was added to the intravenous fluid infusion. Urine was obtained from the bladder, and, at the end of the clearance period,}
blood was sampled from the femoral artery and renal vein using a no. 23-gauge steel needle angled toward the kidney. The study period lasted 60–90 min, during which a single renal clearance was undertaken.

**Measurements of arterial, renal venous, intranephronal, and tissue Po2.** Blood (100 μl) for Po2 was drawn from the femoral artery and renal vein, capped, and kept on ice for measurement of O2 in a blood gas analyzer (Instrumentation Laboratory Synthesis; Boston, MA). Studies for nephron and tissue Po2 utilized a custom-designed and validated ultramicro, coaxial, recessed-tip platinum-iridium glass microelectrode (3, 40, 54). The outer tip (diameter was 3–5 μm) was coated with O2-permeable collodion. It was inserted into superficial tubules or the interstitium of the superficial outer cortex (OC) or was inserted blindly below the surface into the inner cortex (IC) to provide a real-time measure of Po2 using an ultra high impedance picoammeter (pA6000, WIPI, Sarasa, FL).

**Measurements of kidney function and parameters of O2 usage.** The glomerular filtration rate (GFR) was calculated from the clearance of [3H]inulin. The renal plasma flow (RPF) was calculated from the glomerular filtration rate (GFR) was calculated from the clearance of [3H]inulin corrected for the measured renal extraction of [3H]inulin (4). TNa was calculated from the difference between the filtered load of sodium (calculated from the product of GFR and plasma sodium concentration) and the renal sodium excretion (UNaV). QO2 was calculated from the product of renal blood flow (RBF) and arterial-venous O2 difference. The measurements of renal venous O2 content were made in the renal vein after it had exited from the kidney distal to any shunt pathway for O2 (54, 60). The oxygen efficiency for Na+ transport was calculated from the ratio of TNa/UNaV.

**Renal cortex O2− production.** Superoxide was measured by lucigenin-enhanced chemiluminescence (59). Briefly, NADPH (100 μM) was added to the supernatant from the renal cortex in a reaction containing lucigenin (5 μM) and 50 μl protein in the assay buffer just before reading (11, 38). Chemiluminescence was determined over 20 min in a luminometer (Lumat LB 9501; Berthold, Pforzheim, Germany). Basal and NADPH-stimulated O2− production were expressed as nanomoles of O2− per minute per milligram of protein by calibration with SOD-inhibitable cytochrome c reduction (57). Protein was measured using the Bradford method (Bio-Rad; Hercules, CA).

**Renal cortical mRNA and protein expression.** Kidneys were harvested from separate groups of rats (n = 6 per group) after perfusion to wash out blood. The cortex was separated and processed as described (28). The mRNA was extracted and assayed for p22phox by Western blot analysis using a validated antibody (7). Validated antibodies to EC-SOD are not available commercially.

**Statistical methods.** Values are reported as means ± SE. An analysis of variance (ANOVA) assessed the separate and interactive effects of infusions of ANG II and Tempol. Where appropriate, a post hoc Dunnett’s t-test was applied to assess differences between groups. Statistical significance was assumed at P < 0.05.

**RESULTS**

Table 1 shows that there were no significant differences in body weight or hematocrit between the groups. Tempol prevented the reduction in 24-h urine volume with ANG II. Rats infused with ANG II had an increase in MAP and renal vascular resistance (RVR) that were prevented by coinfusion of Tempol (Fig. 1). ANG II reduced the GFR, RPF, and RBF. Surprisingly, Tempol alone also reduced these values and, when infused with ANG II, did not restore the GFR (Table 1).

**Table 1. Body weight, blood pressure, and renal clearance data**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Rats</th>
<th>Body Weight/g</th>
<th>MAP, mmHg</th>
<th>Hct, %</th>
<th>UV, ml/min·100 g−1</th>
<th>GFR, ml/min·100 g−1</th>
<th>RPF, ml/min·100 g−1</th>
<th>FF, %</th>
<th>RBF, ml/min·100 g−1</th>
<th>RVR, mmHg/ml·min−1·100 g−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veh</td>
<td>11</td>
<td>260 ± 11</td>
<td>112 ± 2</td>
<td>42 ± 1</td>
<td>2.0 ± 0.2</td>
<td>0.5 ± 0.05</td>
<td>1.9 ± 0.3</td>
<td>27 ± 2</td>
<td>3.7 ± 0.6</td>
<td>38.7 ± 4.7</td>
</tr>
<tr>
<td>ANG II</td>
<td>8</td>
<td>252 ± 10</td>
<td>146 ± 6</td>
<td>43 ± 1</td>
<td>1.4 ± 0.3</td>
<td>0.42 ± 0.08</td>
<td>1.4 ± 0.2</td>
<td>32 ± 1</td>
<td>2.4 ± 0.4</td>
<td>65.4 ± 6.9</td>
</tr>
<tr>
<td>Tempol</td>
<td>11</td>
<td>274 ± 12</td>
<td>104 ± 3</td>
<td>42 ± 1</td>
<td>1.1 ± 0.2</td>
<td>0.38 ± 0.05</td>
<td>1.1 ± 0.1</td>
<td>33 ± 3</td>
<td>2.4 ± 0.4</td>
<td>44.7 ± 3.1</td>
</tr>
<tr>
<td>ANG II + Tempol</td>
<td>8</td>
<td>249 ± 8</td>
<td>116 ± 5</td>
<td>44 ± 1</td>
<td>2.0 ± 0.3</td>
<td>0.42 ± 0.06</td>
<td>1.6 ± 0.2</td>
<td>28 ± 3</td>
<td>2.6 ± 0.3</td>
<td>44.6 ± 5.2</td>
</tr>
</tbody>
</table>

P value by ANOVA

- ANG II NS
- Tempol NS
- Interaction NS

Values are means ± SE values. Veh, vehicle; ANG II, angiotensin II; MAP, mean arterial pressure; Hct, hematocrit; UV, urine volume; GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; RBF, renal blood flow; RVR, renal vascular resistance; NS, not significant.

**Fig. 1.** Mean ± SE values for mean arterial pressure (A) or renal vascular resistance (B) in groups of rats infused with vehicle (n = 11), angiotensin II (200 ng·kg−1·min−1; n = 8), Tempol (200 nmol·kg−1·min−1; n = 8), or angiotensin II plus Tempol (n = 8). ***P < 0.005 compared with vehicle.
ANG II increased renal cortical NADPH oxidase activity (3.6 ± 0.4 vs. 2.3 ± 0.2 nmol O₂·min⁻¹·mg⁻¹; P < 0.05; Fig. 2). This was prevented by coinfusion of TEMPOL (2.7 ± 0.2 nmol O₂·min⁻¹·mg⁻¹; not significant). ANG II infusion increased the mRNA expression for p22\textsuperscript{phox} by 2.1-fold (2.92 ± 0.22 vs. 4.04 ± 0.20, \(P < 0.01\); Fig. 3). ANG II also increased the protein expression for p22\textsuperscript{phox} by 66% (54.3 ± 6.4 vs. 29.3 ± 5.4 densitometry units, \(P < 0.04\); Fig. 4). There were no significant differences in the expressions of mRNA for p22\textsuperscript{phox} and EC-SOD in animals infused with Tempol alone (3.86 ± 0.22 vs. 4.04 ± 0.20 and \(\Delta C_T\) 4.39 ± 0.12 vs. 4.77 ± 0.24, respectively) and no significant changes in p22\textsuperscript{phox} protein expression. However, coinfusion of Tempol with ANG II abolished the upregulation of mRNA for p22\textsuperscript{phox} (3.66 ± 0.14 vs. 2.92 ± 0.22) and significantly downregulated the protein expression below levels of Veh (11.0 ± 0.6 vs. 29.3 ± 5.4 densitometry units, \(P < 0.02\)). Tempol prevented the ANG II-induced reduction in mRNA for EC-SOD (4.39 ± 0.12 vs. 4.77 ± 0.24).

Parameters of renal oxygen usage and demand obtained under anesthesia are shown in Table 2. There were no differences in arterial O₂ content. ANG II increased renal QO₂ despite a reduced RBF because of an enhanced arteriovenous difference for O₂ across the kidney. Tempol infused alone did not change QO₂ but prevented the increase with ANG II. TNa was reduced by ANG II, Tempol, and ANG II + Tempol because of reductions in GFR. Therefore, the TNa:QO₂ was reduced sharply by ANG II (Fig. 5). This ratio was normalized by coinfusion of Tempol, which had no significant effects when given alone.

Values of PO₂ measured in arterial blood and in the lumen of proximal tubules (PT), distal tubules (DT), and the interstitium of the OC or IC are shown in Table 3 and Fig. 6. There were no changes in arterial PO₂. However, ANG II reduced significantly the PO₂ at all four sites in the kidney cortex. These effects were prevented by coadministration of Tempol, which actually increased the PO₂ above values in vehicle-infused rats in the PT and IC.

**DISCUSSION**

Previous studies in mice (26) and rats (7, 19, 35, 45, 46, 48) have shown that prolonged low-dose infusions of ANG II cause oxidative stress. The main findings of this study are that a slow pressor infusion of ANG II increases renal cortical NADPH oxidase activity, accompanied by increased expression of p22\textsuperscript{phox} and decreased expression of EC-SOD. Whereas this confirms prior findings that AT\(_1\) receptors reduce EC-SOD expression in rats (7) and humans (23), opposite effects are reported in mice (17). ANG II causes hypertension, renal vasoconstriction, and inefficient utilization of renal O₂ for...
vascular hypertension \((62)\).

Tempol is a well-validated spin trap for \(O_2^-\) and generates \(O_2\) (33). Tempol protects cells or tissues from damage due to oxidative stress accompanying cardiac ischemia (18), colitis (25), hyperoxia (42), or radiation (20).

Angiotensin II-induced renal cortical hypoxia was accompanied by inefficient utilization of \(O_2\) for tubular sodium transport. This can be ascribed to oxidative stress because it was prevented by coinfusion of Tempol. As in previous studies in the SHR (60) and the postclip kidney in the 2K,1C model (62), changes in \(T_{Na,Q_2}\) were predictive of the associated changes in \(P_{O_2,renal}\) measured directly in the renal cortex.

Other studies have linked inefficient utilization of \(O_2\) and accompanying tissue hypoxia to oxidative stress. Adler et al. (1) showed that the hearts of aging Fischer rats have oxidative stress due to activation of NAPDH oxidase and reduced control of myocardial \(O_2\) consumption by NO that can be corrected by Tempol. Li et al. (37) demonstrated that mitochondrial SOD knockout mice have diminished defense against local oxidative stress thereby depleting bioactive NO that normally restrains mitochondrial \(O_2\) usage.

Blockade of the \(AT_1\) receptors is the SHR reverses oxidative stress in the kidney and restores a normal blunting of the tubuloglomerular feedback (TGF) response by endogenous NO (63). This suggests that \(AT_1\) receptor activation causes oxidative stress in the renal tubules and interstitium of the cortex that curtails NO bioactivity. Inhibition of NOS in the dog sharply reduces the efficiency with which \(O_2\) is used for tubular \(Na^+\) transport (36). Thus ANG II-induced oxidative stress might reduce \(T_{Na,Q_2}\) by limiting NO bioactivity in the kidney. Indeed, whereas a short-term infusion of ANG II increases renal NO generation (56) and the dependence of RBF on NO, these effects are lost during prolonged ANG II infusions (12). Moreover, the blunting of afferent arteriolar vasoconstriction by NO generated in the afferent arteriole (58, 59) or the macula densa (24) are diminished by prolonged ANG II transport.

Erythropoietin is secreted in response to a fall in renal cortical \(P_{O_2}\). Therefore, our finding that ANG II infusions reduce renal cortical \(P_{O_2}\) may underlie the observations that ANG II infusion normally enhances erythropoietin (16). Moreover, angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) can reverse erythropoietinosis in renal transplant recipients and increase erythropoietin requirements in patients with kidney disease (41). These effects may be secondary to a decrease in renal cortical \(P_{O_2}\), induced by ANG II, or an increase in \(P_{O_2}\) induced by ACEI or ARBs. However, the hematocrit was not change by ANG II infusions.
infusion in the present study. Although, the hematocrit was not changed significantly by ANG II in the present study, it was increased by a higher rate of ANG II infusion in a prior study in mice (26).

Many genes implicated in adverse pathophysiological changes in blood vessels and the kidney during sustained hypertension are activated by the hypoxia-inducible factor (HIF). These include erythropoietin, endothelin-1, vascular endothelial growth factor, tumor necrosis factor-α, transforming growth factor-β, and collagen-1 (49, 55). Therefore, ANG II-induced oxidative stress sufficient to reduce renal PO2 may provide a global mechanism for activation of a number of pathophysiologic processes that together underlie the adverse events that accompany hypertension and prolonged ANG II excess in the kidney and blood vessels.

An interesting finding was that prolonged administration of Tempol reversed both the increased expression of p22\textsuperscript{phox} and the decreased expression of EC-SOD in the kidney cortex. Indeed, p22\textsuperscript{phox} protein expression during ANG II plus Tempol was reduced below basal values. This may explain why Tempol normalizes NADPH oxidase activity in the kidney cortex of ANG II infused rats and reverses oxidative stress in many models of ANG II-induced hypertension (51, 53, 62).

**Perspective.** There are two potentially important implications of this study. First, the finding that Tempol corrects the enhanced NADPH oxidase activity, the enhanced expression of p22\textsuperscript{phox}, and the suppressed expression of EC-SOD in ANG II-infused hypertensive pigs with renovascular hypertension or hypocholesterolemia (8, 9). This extends the “kindling of the fire” hypothesis for the generation of oxidative stress in blood vessels advanced by Harrison and colleagues (22, 34). Second, the findings suggest how oxidative stress during ANG II infusion or hypertension (27) may be held in check by tissue hypoxia. A reduction in PO2 enhances NO bioactivity (50) and reduces O2\textsuperscript{−} generation (44). Therefore, hypoxia may provide the kidney with a means to escape from the vicious cycle whereby oxidative stress causes hypertension and worsens oxidative stress because the accompanying fall in PO2 should limit ongoing O2\textsuperscript{−} generation. However, this postulated benefit would carry with it the threat of activation of hypoxia-dependent genes, many of which have been associated with progressive kidney disease (14, 21).

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**Table 3. Arterial, and intrarenal partial pressures for O2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Rats</th>
<th>Aorta, mmHg</th>
<th>Proximal Tubule, mmHg</th>
<th>Distal Tubule, mmHg</th>
<th>Outer Cortex, mmHg</th>
<th>Inner Cortex, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veh</td>
<td>11</td>
<td>92 ± 5</td>
<td>39 ± 1</td>
<td>40 ± 1</td>
<td>42 ± 2</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>ANG II</td>
<td>8</td>
<td>93 ± 3</td>
<td>34 ± 2</td>
<td>32 ± 2</td>
<td>37 ± 2</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>Tempol</td>
<td>11</td>
<td>99 ± 5</td>
<td>45 ± 2</td>
<td>43 ± 2</td>
<td>44 ± 2</td>
<td>38 ± 2</td>
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<tr>
<td>ANG II + Tempol</td>
<td>8</td>
<td>95 ± 3</td>
<td>42 ± 2</td>
<td>42 ± 2</td>
<td>41 ± 1</td>
<td>36 ± 2</td>
</tr>
</tbody>
</table>

*P value by ANOVA

- ANG II: NS, <0.05, <0.05, <0.05, <0.05
- Tempol: NS, <0.05, NS, NS, NS, <0.05
- Interaction: NS, NS, NS, NS, NS, NS

Values are means ± SE.
REFERENCES


