Roles of Oxidative Stress and AT₁ Receptors in Renal Hemodynamics and Oxygenation in the Postclipped 2K,1C Kidney

William J. Welch, Margarida Mendonca, Shakil Aslam, Christopher S. Wilcox

Abstract—The spontaneously hypertensive rat (SHR) exhibits angiotensin II (Ang II)–dependent oxidative stress and reduced efficiency of renal oxygen usage (QO₂) for tubular sodium transport (TNa). We tested the hypothesis that oxidative stress determines the reduced TNa/QO₂ ratio in the clipped kidney of the early 2-kidney, 1-clip (2K,1C) Ang II–dependent model. One week after sham operation (Sham) or clip placement, 2K,1C rats received for 2 weeks either a vehicle, the superoxide dismutase mimetic tempol (Temp), or candesartan (Cand). Oxidative stress was assessed from excretion of 8-isoprostaglandin F₂α (PGF₂α) and malondialdehyde (MDA) and renal oxygenation from pO₂ in the renal cortex and from the ratio of calculated TNa and QO₂ values. The mean arterial pressure (MAP) of Sham (113±6 mm Hg) was increased in 2K,1C vehicle-treated rats (148±4 mm Hg), but both Temp and Cand restored MAP to Sham levels. The excretions of 8-iso-PGF₂α and MDA were higher in 2K,1C vehicle-treated rats compared with Sham and were normalized by Temp. The pO₂ of Sham (42±2 mm Hg) was lower in 2K,1C vehicle-treated animals (28±2 mm Hg). This was restored to Sham values by Temp (36±3 mm Hg) but not by Cand (28±2 mm Hg). The TNa/QO₂ of Sham (12.9±1.6) was reduced in 2K,1C vehicle-treated rats (9.7±2.8) and was restored to Sham values by Temp (13.7±2.5) but not by Cand (7.5±1.6). We conclude that the correction of oxidative stress in the 2K,1C model partially corrects renal cortical hypoxia and inefficient utilization of O₂ for Na⁺ transport, independent of the fall in blood pressure. (Hypertension. 2003;41[part 2]:692-696.)

Key Words: hypertension, renovascular ■ Goldblatt hypertension ■ renal artery ■ nitric oxide

Renal oxygen consumption (QO₂) is closely related to the energy required for sodium transport (TNa). Classic studies have established that variations in sodium delivery and hence, transport, over a broad range are matched by proportionate changes in QO₂. Prevention of glomerular and hence, transport, over a broad range are matched by studies have established that variations in sodium delivery

Recent studies have reported that the TNa/QO₂ ratio is variable. Laycock and associates showed that the TNa/QO₂ in the dog kidney was reduced by approximately 50% during inhibition of nitric oxide (NO) synthase with L-nitroarginine. We showed a similar reduction in TNa/QO₂ in kidneys from spontaneously hypertensive rats (SHR). The SHR is a model of reduced renal NO bioactivity associated with increased superoxide radical (O₂⁻). There is a complex interrelation between NO, O₂⁻, and PO₂ or O₂ usage in the tissues. In pulmonary arteries and vascular smooth muscle cells, both chronic hypoxia and hyperoxia can enhance O₂⁻ levels. Increased O₂⁻ interacts with NO, which reduces its bioactivity and produces peroxynitrite. Nevertheless, we detected a reduced renal cortical PO₂ in the SHR, which we attributed to inefficient utilization of O₂ for TNa as a consequence of functional NO deficiency during oxidative stress. We found also that the renal cortical hypoxia and reduction in PO₂ in the SHR could be corrected by prolonged administration of the angiotensin receptor antagonist candesartan (Cand) but not by equally effective antihypertensive therapy with agents that do not block the renin-angiotensin system. Moreover, we found that Cand also restored NO bioactivity in the SHR kidney. Angiotensin II (Ang II) stimulates the expression of NADPH oxidase. We concluded that the reduced NO bioactivity and inefficient utilization of O₂ in the SHR kidney could have been secondary to oxidative stress. The present study was designed to test this hypothesis in the early phase of 2-kidney, 1-clip (2K,1C) Goldblatt hypertension. The 2K,1C is a pathophysiological model of Ang II–dependent hypertension, which suppresses function in the clipped kidney in response to oxidative stress.
to the induced renal artery stenosis. One week after the renal artery clip was placed, rats were treated with the superoxide dismutase mimetic tempol (Temp) to reduce O₂− or with Cand to lower blood pressure equivalently.

Methods

These studies were performed under guidelines recommended by the National Institutes of Health and were approved by the Georgetown University Animal Care and Use Committee. Young male Sprague-Dawley rats (80 to 100 g) were anesthetized with isoflurane (0.5% to 1.5%). A silver clip (0.2 mm) was placed around the left renal artery (2K,1C). Sham animals (Sham) were prepared similarly without clip placement. One week thereafter, the 2K,1C rats received for 2 weeks vehicle (Veh, n = 8), Temp (10 mg · kg⁻¹ · min⁻¹ via osmotic minipump, n = 8), or Cand (10 mg · kg⁻¹ · day⁻¹ added to drinking water, n = 9). On day 13 of drug administration, the rats were placed in metabolic cages for a 24-hour urine collection. Whole-body oxidative stress was assessed by the excretion of 8-isoprostaglandin F₂α (8-iso-PGF₂α) and malondialdehyde (MDA) from both kidneys. MDA was measured by an ELISA (Cayman Chemical, Ann Arbor, Mich) on extracted 24-hour samples. MDA was measured by chemical concentration of thiobarbituric acid-reactive substances.

On the following day, rats were anesthetized with Inactin (100 mg/kg IP; Research Biochemicals Inc) and prepared for measurements of mean arterial pressure (MAP), renal function, and renal pO₂, as described previously. To assess renal function in each kidney, urine was collected from the left ureter by placement of a catheter connected to an ultra-high-impedence picoammeter (pA6000; World Precision Instruments). The analog output was digitized and displayed on a computer with an acquisition system (MacLab 4A; AD Instruments). The electrode provides a stable, linear output in response to pO₂ from 5 to 500 mm Hg. Before use, the electrode was calibrated to 4 different levels of O₂. The electrode was inserted into the clipped kidney only and placed either in the outer or inner (advanced 2.0 mm) cortex.

Statistics were assessed by ANOVA, with a post hoc Dunnett’s test to determine differences. Significance was determined at P < 0.05.

Results

All rats grew at normal rates. The body weights at the end of treatment did not differ between groups (Table 1). MAP measured under anesthesia was higher (P < 0.001) in 2K,1C, Veh compared with Sham and was normalized by both Temp and Cand. The clipped kidney weight was lower in 2K,1C Veh and Cand compared with Sham but was not different from Sham in 2K,1C Temp. The right (unclipped) kidney weight was not different among groups. However, the ratio of left to right kidney weight was decreased in 2K,1C Veh compared with Sham and normalized in 2K,1C Temp rats (Table 1). This ratio was further increased in 2K,1C Cand.

As shown in Table 1, the GFR, factored by kidney weight, was reduced in the postclip kidney of 2K,1C, Veh compared with Sham. The low GFR associated with clipping was increased significantly in 2K,1C by Temp but not by Cand. Compared with Sham, the RPF was lower in 2K,1C Veh and was not changed by Cand or Temp. The filtration fraction was similar to Sham in 2K,1C Veh and 2K,1C Temp. However, the filtration fraction was reduced by Cand.

As shown in Figure 1, the excretion of 8-iso-PGF₂α was higher (P < 0.05) in 2K,1C Veh compared with Sham. Treatment with Temp (P < 0.05) or Cand (P < 0.05) reduced 8-iso-PGF₂α excretion to a level comparable to those in Sham. The excretion of MDA also was higher (P < 0.05) in 2K,1C Veh compared with Sham. However, whereas Temp reduced this (P < 0.05) to a level comparable to that in Sham, Cand had no effect. Because the urine samples represent excretion from both kidneys, these values may not reflect the true level of oxidative stress in the clipped kidney.

O₂ extraction, as shown by the difference in renal arterial and venous oxygen content, was elevated significantly in 2K,1C Veh. This was not changed by Temp or Cand (Table 2). However, O₂ extraction in the 2K,1C Temp group was not different from Sham. There were no differences in O₂ usage between groups. Changes in TNa reflected the pattern of changes in GFR.

The O₂ efficiency for Na⁺ transport in the clipped kidney is shown in Figure 2. The TNa/O₂ of Sham was 12.9 ± 1.6 (μmol of Na⁺ transported per μmol of O₂ consumed). This was reduced in 2K,1C Veh 9.7 to 1.8 (P < 0.01) because of a sharp fall in TNa with relative preservation of QO₂. The ratio was restored to Sham values in 2K,1C given Temp (13.7 ± 2.5)
because the reduction in $T_{Na}$ was matched by an equivalent reduction in $Q_{O2}$. However, the $T_{Na}:Q_{O2}$ of 2K,1C given Cand was reduced even further ($7.5\pm1.6$).

The $pO_{2}$ in the inner and outer cortex of the clipped kidney is shown in Figure 3. In Sham animals, the $pO_{2}$ in the outer and inner cortex averaged $42\pm2$ and $33\pm2$ mm Hg, respectively. The $pO_{2}$ in the outer cortex was reduced by $31\%$ and in the inner cortex by $60\%$ in 2K,1C Veh. Whereas 2K,1C rats given Temp had $pO_{2}$ values equivalent to those in Sham in the outer cortex, these were reduced in the inner cortex. The $pO_{2}$ was not improved by Cand compared with Veh 2K,1C. Indeed, there was a further reduction in $pO_{2}$ in the inner cortex to $11\pm3$ mm Hg.

## Discussion

The main new findings from this study are that the clipped kidney of the early 2K,1C model has an increased use of $O_{2}$ in relation to $T_{Na}$ associated with a sharp reduction of $pO_{2}$ in the outer cortex and especially the inner cortex. These abnormalities are largely prevented by Temp, which also prevented the whole-body oxidative stress associated with the clipping. Although Temp treatment led to a normal blood pressure in the 2K,1C rats, the defects in oxygenation were not corrected by equivalent reduction in blood pressure by Cand. Indeed, Cand led to a further decline in $T_{Na}:Q_{O2}$ and to a reduction in $pO_{2}$ in the inner cortex to $11\pm3$ mm Hg. Moreover, the reduction in the ratio of kidney weight (clipped to unclipped) of $78\%$ in 2K,1C rats given Veh was improved by Temp but worsened by Cand.

The relation between NO and $O_{2}^{-}$ affects both vascular tone and $O_{2}$ consumption. NO at physiological levels can compete with $O_{2}$ for the respiratory chain in mitochondria. Thus, low levels of NO after inhibition of NO synthase enhance $O_{2}$ usage and reduce the levels of oxygen in various tissues, including the kidney. Decreased bioactive NO is found in the kidney in the SHR, which is a model of oxidative stress. In this setting, the low NO in the juxtaglomerular apparatus (JGA) can be ascribed to interaction with $O_{2}^{-}$, because NO bioactivity increased 6-fold after local microper-

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**TABLE 2. Oxygen Extraction and Consumption in the Left Kidney**

<table>
<thead>
<tr>
<th>Group</th>
<th>$(A-V)O_{2}$ (μmol)</th>
<th>$T_{Na}$ (μmol/min)</th>
<th>$Q_{O2}$ (μmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (n=9)</td>
<td>2.1±0.3</td>
<td>154±7</td>
<td>12.2±2.2</td>
</tr>
<tr>
<td>2K,1C Vehicle (n=7)</td>
<td>3.0±0.5*</td>
<td>91±4†</td>
<td>10.2±1.9</td>
</tr>
<tr>
<td>2K,1C Cand (n=8)</td>
<td>3.0±0.4*</td>
<td>78±4†</td>
<td>12.0±2.7</td>
</tr>
<tr>
<td>2K,1C Tempol (n=7)</td>
<td>2.3±0.4</td>
<td>134±9†</td>
<td>9.7±2.0</td>
</tr>
</tbody>
</table>

$(A-V)O_{2}$ indicates the difference between oxygen content in the femoral artery and that in the renal vein; $T_{Na}$, sodium transported; and $Q_{O2}$, oxygen consumption.

*P<0.05, †P<0.001 compared with Sham; ‡P<0.001 compared with vehicle.

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**Figure 1.** Mean±SEM values for 24-hour excretion of 8-isoprostaglandin $F_{2\alpha}$ (8-isoPGF$_{2\alpha}$, left) and malondialdehyde (MDA) (right). Veh indicates vehicle; Cand, candesartan; Temp, tempol.

**Figure 2.** Mean±SEM calculated values for tubular sodium transport relative ($T_{Na}$) to renal oxygen usage ($Q_{O2}$) in the left kidney. Veh indicates vehicle; Cand, candesartan; Temp, tempol.
fusion of Temp to metabolize $O_2$\textsuperscript{−}.\textsuperscript{8} Thus, we propose that the reduced $T_{Na}:Q_{O2}$ and the reduced $pO_2$ of the SHR kidney might be secondary to NO deficiency owing to interaction with $O_2$\textsuperscript{−}. However, we cannot rule out additional effects of Temp in the development of renovascular hypertension. Because kidney size was not reduced in this group, it is plausible that the beneficial effects are related to more complete perfusion of the kidney during this typically ischemic phase of this model. However, this effect may also be related to superoxide dismutase activity, because Temp promotes longer bioactivity of NO and would help to maintain stable blood flow.

Further evidence is derived from the results of prolonged blockade of Ang II type I receptors with Cand. Cand corrected oxidative stress, restored NO bioactivity in the JGA, and normalized renal $T_{Na}:Q_{O2}$ and renal $pO_2$ levels in SHR.\textsuperscript{4} Therefore, we elected to study the relation between oxidative stress and oxygenation more directly in the present series in a model of extreme Ang II action in the clipped kidney of 2K,1C rats. Previous studies in the 2K,1C pig\textsuperscript{15} and rat\textsuperscript{16} showed that increased oxidative stress was associated with high renin levels in the cortex in the early phase. We have consistently observed low $pO_2$ in the cortex of hypertensive kidneys, which also demonstrate high oxidative stress and deficient NO. However, direct supporting evidence of this relation is currently unavailable. The finding that Temp corrected oxidative stress, reduced renal cortical $pO_2$, and reduced renal oxygen usage for $T_{Na}$ provides direct evidence linking defective renal oxygenation to oxidative stress. Because $T_{Na}:Q_{O2}$ may be dependent on available $O_2$, the effect on superoxide and NO production may suggest that this parameter is linked to redox capacity within the kidney.

Studies in patients with renovascular hypertension due to unilateral renal artery stenosis show enhanced markers for oxidative stress and decreased renal excretion of NO metabolites associated with endothelial dysfunction.\textsuperscript{17} All of these parameters are normalized after correction of the hypertension by renal angioplasty. Our study suggests that the beneficial effect of angioplasty is not due to the reduction in blood pressure, because our previous study detected no benefit from nonspecific antihypertensive treatment in the SHR model and no benefits in the present study in 2K,1C hypertension with Cand. In another study of patients with unilateral renal artery stenosis, $O_2$ saturation was consistently lower in the blood draining from the contralateral compared with the poststenotic kidney.\textsuperscript{18} The authors related the enhanced renal $O_2$ uptake to a reduced filtration fraction, leading to a lower level of Na\textsuperscript{+} reabsorption. However, neither $T_{Na}$ nor $Q_{O2}$ could be assessed in this study. We did not measure the $O_2$ content of the contralateral kidney in our study. Nevertheless, the $O_2$ content of the postclip kidney was already reduced sharply (Table 2). Therefore, if the contralateral kidney has even lower renal venous $O_2$ content, as anticipated from this clinical study, then it must likely also have profound defects of oxygenation.

Because treatment trials of angioplasty of the stenotic renal artery in human renovascular hypertension have been quite disappointing, attention has focused on the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. However, although these drugs can reduce blood pressure quite effectively, they can cause a further loss of GFR and size in the poststenotic kidney. This has limited enthusiasm for their use in this condition. The results in this model of acute 2K,1C hypertension may not be fully applicable to chronic renovascular hypertension in humans. Nevertheless, blocking of the angiotensin AT\textsubscript{1} receptor in the 2K,1C model reduced the GFR and size of the postclipped kidney. In contrast, we observed that scavenging superoxide with Temp not only corrected hypertension but also normalized the GFR and the relative size of the 2 kidneys. Importantly, the angiotensin receptor blocker led to a further deterioration in $T_{Na}:Q_{O2}$ and to a profound fall in $pO_2$ in the deep cortex to values as low as 11 mm Hg, whereas these parameters of oxygenation were improved by Temp. This suggests that treatment aimed at correction of oxidative stress could have advantages over current therapy for renovascular hypertension.

In summary, we found that in renovascular hypertension generated by clipping of the left renal artery, oxidative stress was increased both systemically and in the kidney. In the clipped kidney, GFR, RBF, and the efficient use of oxygen were reduced, concomitant with hypertension. Correction of hypertension by Cand did not improve renal function or oxygen efficiency in the clipped kidney. Reduction of hypertension by Temp led to normalized renal function and oxygen efficiency in the clipped kidney. The renal tissue $pO_2$ in both the outer and inner cortex of the left kidney was also reduced by clipping. The tissue $pO_2$ was unaffected by Cand but was normalized by Temp. We conclude that suppression of oxidative stress in this model partially corrects renal cortical hypoxia and inefficient usage of $O_2$ for sodium transport.

**Perspectives**

This study extends the observations that many of the physiological consequences of elevated Ang II are mediated by superoxide. Renal function and the efficient use of oxygen in
the kidney are both suppressed during high superoxide conditions of renovascular hypertension. This suggests that the levels of oxygen in the kidney are dependent on the utilization of oxygen for the work of sodium transport. Therefore, the relation of oxygen with the important metabolites NO and superoxide may be partially dependent on energy requirements. This represents novel regulation of a potentially potent vasoactive family.

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References
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