Impaired Regulation of Renal Oxygen Consumption in Spontaneously Hypertensive Rats

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Abstract. Abnormalities of nitric oxide (NO) and oxygen radical synthesis and of oxygen consumption have been described in the spontaneously hypertensive rat (SHR) and may contribute to the pathogenesis of hypertension. NO plays a role in the regulation of renal oxygen consumption in normal kidney, so the response of renal cortical oxygen consumption to stimulators of NO production before and after the addition of the superoxide scavenging agent tempol (4-hydroxy-2,2,6,6-tetramethyl piperidine-1-oxyl) was studied. Baseline cortical oxygen consumption was similar in SHR and Wistar-Kyoto (WKY) rats (SHR: 600 ± 55 nmol O2/min per g, WKY: 611 ± 51 nmol O2/min per g, P > 0.05). Addition of bradykinin, enalaprilat, and amlodipine decreased oxygen consumption significantly less in SHR than WKY (SHR: bradykinin −13.9 ± 1.9%, enalaprilat −15.3 ± 1.6%, amlodipine −11.9 ± 0.7%; WKY: bradykinin −22.8 ± 1.0%, enalaprilat −24.1 ± 2.0%, amlodipine −20.7 ± 2.3%; P < 0.05), consistent with less NO effect in SHR. Addition of tempol reversed the defects in responsiveness to enalaprilat and amlodipine, suggesting that inactivation of NO by superoxide contributes to decreased NO availability. The response to an NO donor was similar in both groups and was unaffected by the addition of tempol. These results demonstrate that NO availability in the kidney is decreased in SHR, resulting in increased oxygen consumption. This effect is due to enhanced production of superoxide in SHR. By lowering intrarenal oxygen levels, reduced NO may contribute to susceptibility to injury and renal fibrosis. Increasing NO production, decreasing oxidant stress, or both might prevent these changes by improving renal oxygenation.

Nitric oxide (NO) plays an important role in regulation of vascular tone. Absence of the NO generating enzyme endothelial nitric oxide synthase (eNOS) or impairment of NO production leads to hypertension in animal models and can increase vascular tone in humans (1–4). In a model of genetic hypertension in the rat, the spontaneously hypertensive rat (SHR), abnormalities in synthesis of NO, expression of the NO-synthesizing enzymes, or both have been described both in vitro and in vivo, but with conflicting results. Thus, several studies have been performed to provide evidence of impaired vasodilation in response to acetylcholine, an effect mediated by endothelium-derived relaxing factor or NO, decreased eNOS expression, or decreased NO synthesis in SHR (5–11). These abnormalities are present as early as 5 wk of age, a period before the development of hypertension (7). However, several of these studies have shown a decreased effect of NO rather than direct evidence of decreased production.

On the other hand, evidence of increased expression of NO synthesizing enzymes (eNOS and inducible NO synthase), increased NO production, or both have also been noted (12–18), both before and after the onset of hypertension. One possible explanation for this discrepancy is inactivation of NO, explaining increased production but less effect in SHR. NO is inactivated by reaction with superoxide (O2·−) to produce peroxynitrite and increased production of superoxide has been demonstrated in SHR (13,19–21). Manipulations that reduce superoxide production have also been shown to lower BP in these animals (19–21).

NO also modulates oxygen consumption in the whole animal and in isolated tissues, such as heart and kidney, with an inverse relationship between NO production and oxygen consumption (22–24). We have previously shown that NO production by the eNOS isoenzyme in the kidney is responsible for regulation of renal oxygen consumption (25). Whole-body oxygen consumption is increased in SHR, an observation consistent with a decreased effect of NO (26). More recently, studies in SHR have demonstrated a relative increase in oxygen consumption in kidney when compared with sodium reabsorption, indicating relative inefficiency of oxygen usage in these animals (27). Therefore, we hypothesized that NO regulation of oxygen consumption is impaired in the kidney of SHR and that interference with superoxide production would restore the normal effects of agonists of NO production on renal oxygen consumption.

Materials and Methods

Reagents

Bradykinin, enalaprilat, S-nitroso-N-acetylpenicillamine (SNAP), N-nitro-l-arginine methyl ester (l-NAME), tempol (4-hydroxy-2,2,6,6-tetramethyl piperidine-1-oxyl), sodium succinate, and sodium cyanide were purchased from Sigma Chemical Company (St. Louis, MO). Amlodipine was provided by Pfizer (Groton, CT).
Animals
Male SHR (n = 6) and Wistar-Kyoto (WKY; n = 6) rats were purchased from Taconic Farms, Inc. (Germantown, NY) when they were 10 wk old and studied after 1 wk of acclimatization. Rats were maintained on a standard rat chow with 0.4% sodium content (Laboratory Rodent Diet, Richmond, IN). The rats were allowed free access to food and water until the day they were killed. Before they were killed, BP and heart rate were measured with a noninvasive BP monitor (Columbus Instruments, Columbus, OH), and blood was drawn from the femoral vein for measurement of hemoglobin, blood urea nitrogen, and creatinine assessment. After the animals were killed, the left kidneys were removed, decapsulated, and weighed.

Preparation of Kidney Tissue Slices and Measurement of O₂ Consumption
Thin slices of renal cortex (approximately 1 mm, weight 10 to 20 mg) were prepared and incubated in Krebs bicarbonate solution (containing, in mmol/L, NaCl 118, KCl 4.7, CaCl₂ 1.5, NaHCO₃ 25, KH₂PO₄ 1.2, MgSO₄ 1.1 and glucose 5.6, pH 7.4) bubbled with 21% O₂/5% CO₂/74% N₂ at 37°C for 2 h. At the end of incubation, each piece of tissue was placed in a stirred chamber with 3 ml of air-saturated Krebs bicarbonate solution containing 10 mmol/L N₂-hydroxyethylpiperazine-N'-2-ethane sulfonic acid and 5.6 mmol/L glucose (pH 7.4). The chamber was sealed with a Clark-type platinum O₂ electrode (Yellow Springs Instruments, Yellow Springs, OH). O₂ consumption was measured polarographically with an O₂ monitor (model YSI 5300) connected to a linear chart recorder (model 1202, Barnstead/Thermolyne Corp., Dubuque, IA). Dose-response curves of the effect of different agonists on kidney O₂ consumption were then measured. Succinate (10⁻³ mol/L) and then sodium cyanide (10⁻³ mol/L) were added at the end of each experiment to confirm that changes in O₂ consumption originated from mitochondrial respiration.

Renal cortical O₂ consumption is calculated as the rate of decrease in O₂ concentration, assuming an initial O₂ concentration of 224 mmol/ml (calculated from O₂ solubility at 37°C and 1 atm pressure) and is expressed as nanomoles of O₂ consumed per minute per gram of tissue. O₂ consumption due to the electrode is less than 5% of that in O₂ concentration, assuming an initial O₂ concentration of 224 mmol/ml (calculated from O₂ solubility at 37°C and 1 atm pressure).

Effect of Agonists on O₂ Consumption
Bradykinin or enalaprilat at concentrations of 10⁻⁷ to 10⁻⁴ mol/L, or amlodipine at concentrations of 10⁻⁷ to 10⁻⁵ mol/L, were added in a cumulative concentration-dependent manner. They were used to measure the effects of stimulation of endogenous NO production on renal O₂ uptake. The response to these drugs was also examined after preincubation with L-NAME (10⁻³ mol/L). Each condition was tested in six rats from each group.

Effect of NO Donor on O₂ Consumption
SNAP at concentrations of 10⁻⁷ to 10⁻⁴ mol/L was added in a cumulative concentration-dependent manner to assess the effects of exogenous NO on renal cortical O₂ uptake. The response to SNAP was also examined after preincubation with L-NAME (10⁻³ mol/L). Each condition was tested in six rats from each group.

Statistical Analyses
All data are expressed as mean ± SEM. Statistical analyses of baseline O₂ consumption were performed by t test. Changes in O₂ consumption caused by drug treatment were analyzed by two-way ANOVA followed by multiple comparisons by the Tukey test (SigmaStat; SPSS, Chicago, IL). Statistical significance was achieved at P < 0.05.

Results
Baseline characteristics of the rats used in these studies are listed in Table 1. Kidney/body weight, heart rate, hemoglobin levels, and creatinine levels were similar in the two groups. SHR rats had significantly higher systolic, diastolic, and mean arterial pressures (P < 0.01).

Baseline Renal Cortical O₂ Consumption
Baseline renal cortical O₂ consumption was similar in the two groups (WKY: 611 ± 51 nmol O₂/min per g, n = 6; SHR: 600 ± 55 nmol O₂/min per g, n = 6, P > 0.05). Addition of the NOS inhibitor L-NAME (10⁻⁷ mol/L) did not significantly alter O₂ consumption (WKY: 638 ± 53 nmol O₂/min per g, n = 6; SHR: 634 ± 66 nmol O₂/min per g, n = 6, P > 0.05).

Effect of Bradykinin on Renal O₂ Consumption
Cumulative doses of bradykinin (10⁻⁷ to 10⁻⁴ mol/L) produced significant, concentration-dependent decreases of renal cortical O₂ consumption in WKY and SHR rats (WKY: from -2.1 ± 1.1% to -22.8 ± 1.0%, n = 6; SHR: from -0.0 ± 0% to -13.9 ± 1.9% n = 6). The depression of renal cortical O₂ consumption by bradykinin was significantly less in SHR than WKY rats at 10⁻⁶ to 10⁻⁴ mol/L of bradykinin (P < 0.05 for each comparison) (Figure 1). Addition of L-NAME significantly attenuated bradykinin induced decreases in O₂ consumption at 10⁻⁶ to 10⁻⁴ mol/L of bradykinin in WKY rats (from -0.5 ± 0.5% to -12.0 ± 1.4%, n = 6; P < 0.05 for each comparison), demonstrating the importance of NO synthesis in the effect of bradykinin (Figure 1). In SHR rats, addition of L-NAME had a smaller and NS effect in reversing the action of bradykinin on renal O₂ consumption from -0 ±

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WKY (n = 10)</th>
<th>SHR (n = 10)</th>
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<tbody>
<tr>
<td>Kidney/body weight ratio</td>
<td>0.34 ± 0.01</td>
<td>0.34 ± 0.01</td>
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<tr>
<td>Systolic BP (mmHg)</td>
<td>133.0 ± 3.2</td>
<td>188.4 ± 3.5b</td>
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<td>Diastolic BP (mmHg)</td>
<td>107.9 ± 2.6</td>
<td>147.7 ± 3.2b</td>
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<td>Mean arterial pressure (mmHg)</td>
<td>117.2 ± 2.4</td>
<td>161.2 ± 3.2b</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>402 ± 16</td>
<td>403 ± 9</td>
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<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.1 ± 0.5</td>
<td>13.0 ± 0.3</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>0.55 ± 0.02</td>
<td>0.58 ± 0.03</td>
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* Data are mean ± SEM. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

b P < 0.01.
Effect of Enalaprilat on Renal O₂ Consumption

The angiotensin-converting enzyme inhibitor, enalaprilat (10⁻⁷ to 10⁻⁴ mol/L), which stimulates endogenous NO production, similarly caused concentration dependent decreases in renal cortical O₂ consumption in WKY and SHR rats (WKY: from −3.5 ± 1.0% to −24.1 ± 2.0%, n = 6; SHR: from −0.4 ± 0.4% to −15.3 ± 1.6% n = 6). The depression of cortical O₂ consumption was significantly less in SHR than WKY rats at all doses of enalaprilat (P < 0.05 for each comparison) (Figure 2). Addition of L-NAME significantly attenuated the effect of enalaprilat at all doses in WKY rats (from −0.0 ± 0.0% to −13.8 ± 2.1% n = 6, P < 0.05 versus each comparison without L-NAME) but had no significant effect in SHR rats (−0.0 ± 0.0% to −13.8 ± 2.1% n = 6, P > 0.05).

Effect of Amlodipine on Renal O₂ Consumption

Amlodipine (10⁻⁷ to 10⁻⁵ mol/L), which stimulates renal NO production, significantly decreased renal cortical O₂ consumption in WKY and SHR rats (WKY: from −4.5 ± 1.4% to −20.7 ± 2.3%, n = 6; SHR: from −1.9 ± 0.6% to −11.9 ± 0.7% n = 6). The depression of cortical O₂ consumption was significantly less in SHR than WKY rats at all doses of amldopine (P < 0.05 for each comparison) (Figure 3). Addition of L-NAME again significantly attenuated the effect of amlodipine at all doses only in the WKY rats (WKY: from −1.0 ± 0.6% to −13.0 ± 1.6%, n = 6, P < 0.05 versus each comparison without L-NAME; SHR: from −0.5 ± 0.5% to −10.0 ± 2.5% n = 6, P > 0.05).

Figure 1. Effect of cumulative doses of bradykinin on renal cortical O₂ consumption in Wistar-Kyoto rats (WKY) (A) and spontaneously hypertensive rats (SHR) (B) in the absence (solid circles) or presence (open circles) of N-nitro-L-arginine methyl ester (L-NAME). Bradykinin caused dose-dependent decreases in O₂ consumption in both groups, although the decrease in SHR rats was significantly less than in WKY rats. This effect was significantly attenuated by the addition of the NOS inhibitor L-NAME (open circles) only in WKY rats. *, P < 0.05 versus WKY rats in the presence of L-NAME and SHR rats in the absence of L-NAME.

Figure 2. Effect of cumulative doses of enalaprilat on renal cortical O₂ consumption in Wistar-Kyoto (WKY) (A) and spontaneously hypertensive rat (SHR) (B) rats in the absence (solid circles) or presence (open circles) of N-nitro-L-arginine methyl ester (L-NAME). Enalaprilat caused dose-dependent decreases in O₂ consumption in both groups, although the decrease in SHR rats was significantly less than in WKY rats at all doses of enalaprilat. This effect was significantly attenuated by the addition of L-NAME (open circles) only in WKY rats. *, P < 0.05 versus WKY rats in the presence of L-NAME and SHR rats in the absence of L-NAME.
Effect of an NO Donor (SNAP) on Renal O$_2$ Consumption

Administration of cumulative doses of the NO donor SNAP ($10^{-7}$ to $10^{-4}$ mol/L) decreased renal cortical O$_2$ consumption in WKY and SHR rats to similar degrees (WKY: from $0.0 \pm 0.0\%$ to $-41.7 \pm 1.9\%$, $n = 6$; SHR: from $-0.0 \pm 0.0\%$ to $-39.7 \pm 1.7\%$, $n = 6$, $P > 0.05$ at all doses), demonstrating no inherent difference between the strains in responsiveness of O$_2$ consumption to NO (Figure 4). Addition of L-NAME had no effect on the response to SNAP (data not shown).

Effect of Oxygen Radical Scavenging with Tempol on Renal O$_2$ Consumption

Elevated levels of oxygen radicals have been noted in SHR rats and have been postulated to decrease availability of NO. Therefore, we added the superoxide dismutase mimetic tempol ($10^{-3}$ mol/L) to some incubations to see the effect of decreasing superoxide production on the response of renal cortical O$_2$ consumption to stimulators of NO production. Enalaprilat again decreased O$_2$ consumption significantly more in WKY as compared with SHR rats (Figure 5A). Addition of tempol significantly restored the suppression of O$_2$ consumption by enalaprilat ($10^{-6}$-$10^{-4}$ mol/L) in SHR rats to a level that was not different from WKY rats. Suppression of O$_2$ consumption by amlodipine ($10^{-5}$ mol/L) was also significantly enhanced (Figure 5B). Tempol had no effect on the responsiveness of WKY rats to enalaprilat or amlodipine at any dose and had no effect on the responsiveness or either group to SNAP (Figure 5C).

Discussion

The results presented here provide evidence for the hypothesis that regulation of renal oxygen consumption by NO is impaired in SHR and that this impairment is due to ineffective NO production as a result of NO inactivation by superoxide. Bradykinin, enalaprilat, and amlodipine, all stimulators of NO production, decreased renal cortical oxygen consumption in a dose-dependent manner in WKY rats, similar to results previ-

Figure 3. Effect of cumulative doses of amlodipine on renal cortical O$_2$ consumption in Wistar-Kyoto (WKY) (A) and spontaneously hypertensive rat (SHR) (B) rats in the absence (solid circles) or presence (open circles) of N-nitro-L-arginine methyl ester (L-NAME). Amlodipine caused dose-dependent decreases in O$_2$ consumption in both groups. The decrease in SHR rats was significantly less than in WKY rats at all doses of amlodipine. Addition of L-NAME (open circles) significantly attenuated the effect of amlodipine only in WKY rats. *, $P < 0.05$ versus WKY rats in the presence of L-NAME and SHR rats in the absence of L-NAME.

Figure 4. Effect of cumulative doses of S-nitroso-N-acetylpenicillamine (SNAP) on renal cortical O$_2$ consumption in Wistar-Kyoto (WKY) (A) and spontaneously hypertensive rat (SHR) (B) rats in the absence (solid circles) or presence (open circles) of N-nitro-L-arginine methyl ester (L-NAME). SNAP caused similar dose-dependent decrease in O$_2$ consumption in both groups ($P > 0.05$). Addition of L-NAME did not alter the effect of SNAP (data not shown).
uously reported in normal mice and dogs (25,28). In SHR, there was a significant decrease in the response to all three of these stimulators of endogenous NO production, suggesting a defect in renal NO production. The absence of a difference in basal oxygen consumption between the two groups, despite evidence for a defect in NO production in SHR, is most likely due to the absence of flow in this preparation, which results in little eNOS activation and suggests very low basal NO production. Thus, the difference between the two groups does not become evident until NO production is stimulated.

In the presence of tempol, a superoxide dismutase mimetic that reduces superoxide levels, the response of oxygen consumption in SHR kidney to amlodipine and enalaprilat was restored to levels seen in control rats. Thus, production of NO in the renal cortex in SHR appears to be adequate to produce a similar decrease in oxygen consumption as in controls as long as superoxide production is inhibited. The responsiveness of kidneys from both SHR and WKY to SNAP, and the lack of effect of tempol on the SNAP response suggest that there is no difference in the ability of NO to alter oxygen consumption in the two groups.

The major effect of NO on oxygen consumption occurs through a direct action of NO on mitochondrial respiration (22,29,30). NO binds to several enzymes in the mitochondrial electron transport chain, including aconitase, complex I and II, and cytochrome oxidase (reviewed in [30]). In the kidney, NO may also decrease oxygen consumption through an effect on sodium transport, a major determinant of oxygen usage. NO inhibits thick ascending limb chloride flux, perhaps through an effect on the Na⁺-K⁺-2Cl⁻ transporter, and has also been shown to directly inhibit sodium-potassium ATPase (31–33).

Our results are consistent with and extend observations by others on NO production and the role of superoxide on NO availability in SHR. Both short- and long-term treatment with tempol normalized BP and renal vascular resistance in SHR, an effect that was shown to be dependent on NO synthesis (19,20). Treatment with another antioxidant, lazaroid, also lowered BP in SHR, along with decreases in the renal expression of eNOS and inducible NO synthase (21), an effect suggested to be due to inhibition of NOS synthesis by the newly available NO. In our studies, tempol restored NO inhibition of renal oxygen consumption to normal levels. These results all substantiate the presence of increased oxidant production and decreased NO availability in SHR.

Our results are also interesting in the context of the increased whole-body oxygen consumption observed in SHR (26). In conscious dogs, inhibition of NO synthesis with nitro-L-arginine leads to increased total body oxygen consumption (22). Thus, one explanation for the changes observed in SHR is decreased NO synthesis or availability. More recently, by using an oxygen-sensitive ultramicroelectrode, Welch et al. (27) demonstrated normal total renal oxygen consumption in SHR, but a 43% reduction in tubular reabsorption of sodium, a major determinant of renal oxygen consumption. Thus, the efficiency of oxygen usage for sodium transport is decreased in SHR, an effect similar to that demonstrated in conscious dogs following inhibition of NO synthesis (24). They hypothesized that the reduction in efficiency of oxygen usage might be due to a relative state of NO deficiency. The data presented here substantiate that conjecture.

By regulating oxygen consumption, NO may also play an important role in regulation of intrarenal oxygenation and predisposition to injury. Studies by Brezis et al. (34,35) found intrarenal pO₂ of approximately 46 mmHg in the cortex and 21 to 23 mmHg in the medulla. Studies that used isolated renal tubules have demonstrated a markedly enhanced effect of NO on inhibition of oxygen consumption as pO₂ drops to these levels (36). Under the hypoxic conditions present in the cortex, and even more so in the medulla, the effect of NO, or the lack thereof, on oxygen consumption will be magnified. Inhibition of NO synthesis with 1-NAME decreases medullary pO₂ (34),
an effect postulated to be due to decreased blood flow, but also now explained by increased oxygen consumption in the face of low NO. t-NAME also increased hypoxic injury to tubules in the medulla in rats, an effect reversed by infusion of the NO donor nitroprusside (34), again an effect mediated either by nitroprusside induced vasodilation or improved oxygenation through inhibition of oxygen consumption by NO. Similarly, in congestive heart failure, in which we have demonstrated decreased NO effect in the kidney (28), outer medullary hypoxic damage is worsened following injection of indomethacin and t-NAME (37).

Intrarenal pO2 is also lower in SHR than WKY rats in the proximal and distal tubules and in the outer and deep cortex (27), which can be explained by increased oxygen consumption related to decreased NO availability. NO production in the kidney may also be impaired in other disease states, including diabetes, other models of hypertension, aging, and chronic renal insufficiency (38–41). Thus, increased sensitivity to renal insults might be expected in these states, a hypothesis consistent with clinical observations. More importantly, evidence has recently accumulated that intrarenal hypoxia may predispose to fibrosis, leading to progressive loss of renal function.

Hypoxia regulates the production of a broad spectrum of growth factors, hormones, matrix components, proteases, and protease inhibitors (reviewed in [42]). Exposure of human proximal tubular epithelial cells to hypoxia stimulates collagen production, decreases gelatinase A production, and increases production of tissue inhibitor of metalloproteinase-1, changes that would favor matrix accumulation (43). In human renal fibroblasts, hypoxia also produces changes favoring matrix accumulation, an effect at least partially mediated through the hypoxia-inducible transcription factor 1 (44). Fine et al. (42) have hypothesized that treatments such as angiotensin-converting enzyme inhibitors, which slow progression of renal disease, may work not only by decreasing proteinuria but by increasing microvascular flow in the interstitium and raising intrarenal pO2. Our data suggest that angiotensin-converting enzyme inhibitors, such as enalapril, might also increase intrarenal oxygen tension by decreasing oxygen consumption through stimulation of NO synthesis. Further studies of these mechanisms could lead to new treatments to slow the progression of renal disease.

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