Oxidant Stress Leads to Impaired Regulation of Renal Cortical Oxygen Consumption by Nitric Oxide in the Aging Kidney

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Abstract. Structural and functional changes occur in the kidney with aging. Previous studies have suggested that loss of nitric oxide production contributes to these changes. The authors therefore explored regulation of renal cortical oxygen consumption, a nitric oxide mediated effect, in tissue from Fischer 344 rats at different ages (4, 13, and 23 mo) to characterize changes in renal nitric oxide production with age. Bradykinin, enalaprilat, and amlodipine significantly suppressed cortical oxygen consumption in 4-mo-old rats (bradykinin: −2.5 ± 0.9% to −21 ± 1.5%; enalaprilat: −0.7 ± 0.5% to −26 ± 1.2%; amlodipine: −1.3 ± 0.9% to −18 ± 1.2%; P < 0.05). Similar results were obtained in 13-mo-old animals. However, in 23-mo-old animals, the responses to bradykinin and enalaprilat were attenuated (bradykinin: 0 ± 0% to −13 ± 0.9%; enalaprilat: −0.3 ± 0.3% to −17 ± 2.1%; P < 0.05), whereas the response to an NO donor was unaffected, suggesting decreased bioavailability of NO. Addition of the superoxide radical scavenger tempol restored the ability of bradykinin, enalaprilat, and amlodipine to suppress oxygen consumption in tissue from 23-mo-old animals to levels seen in younger animals, suggesting NO destruction by superoxide as the reason for decreased NO availability. Apocynin, an inhibitor of NAD(P)H oxidase, similarly restored the ability of all three drugs to suppress oxygen consumption, suggesting NAD(P)H oxidase as the enzyme responsible for enhanced superoxide production in aging. Levels of eNOS protein, assessed by immunoblotting, did not change significantly with age. These results suggest that NO availability is decreased in the aging kidney and that this is due to scavenging of NO by superoxide produced by NAD(P)H oxidase. Oxidant stress, by depleting NO, may contribute to the structural and hemodynamic changes characteristic of the aging kidney.

The aging kidney is characterized by loss of renal mass, arterial and arteriolar hyalinosis, an increased number of sclerotic glomeruli, loss of tubules, and interstitial fibrosis (1). Functionally, there is a trend toward decreased renal blood flow and glomerular filtration with a rise in filtration fraction and impairment of tubular functions such as sodium handling, concentration/dilution, and acidification (1). Nitric oxide (NO), a potent vasodilator, is an important regulator of renal hemodynamics, as well as being involved in regulation of sodium handling (2), leading to interest in changes in NO production in the aging kidney as a contributing factor to altered renal function. Studies in aged rats have found evidence of decreased total body NO production, as manifested by decreased excretion of nitrates plus nitrites (NOx) in the urine and decreased vascular responsiveness to acetylcholine, supporting decreased vascular NO production (3,4). However, intact production of NO by renal vasculature was found in another study of aging rats despite decreased urinary NOx excretion (5), and NO may become more important to maintenance of renal perfusion during aging (5,6). In studies of aging humans, decreased urinary excretion of NOx was not found, although the authors point out the questionable value of such measurements in assessing renal NO activity (7). In aging male, but not female, Sprague-Dawley rats, decreases in total nitric oxide synthase (NOS) activity, as well as endothelial and neuronal NOS (eNOS and nNOS) levels, were found in the kidney (8), suggesting that renal NO production might be impaired in older males.

Another function of NO in the kidney is regulation of renal oxygen consumption through an inhibitory effect on mitochondrial respiration (9). We have shown that this is mediated via NO produced by eNOS (10). Regulation of renal oxygen consumption by NO is impaired in heart failure, the spontaneously hypertensive rat (SHR), and diabetes (references 11 and 12 and unpublished observations). Particularly in the SHR, others have shown relative inefficiency of oxygen usage in the kidney, leading to lowering of intrarenal pO2 (13). We have provided evidence that this is due to decreased NO availability owing to scavenging by superoxide (12). Worsening of renal hypoxia by decreased NO production, as well as loss of the vasodilatory action of NO, could play a role in increasing
susceptibility to ischemic injury and progression of renal disease (14,15).

To explore intrarenal NO production in aging, we examined renal cortical oxygen consumption in kidney from aging Fischer 344 rats. We hypothesized that there would be impaired production of NO in aging leading to decreased regulation of oxygen consumption by stimulators of NO synthesis. We further hypothesized that oxygen radicals contribute to the defects of NO biologic activity in the aging kidney and thus explored the effects of a superoxide scavenger and an inhibitor of superoxide production.

Materials and Methods

Reagents

Bradykinin, enalaprilat, s-nitroso-N-acetylpenicillamine (SNAP), N-nitro-L-arginine methyl ester (L-NAME), sodium succinate, sodium cyanide, 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (tempol), and apocynin were purchased from Sigma-Aldrich Co. (St. Louis, MO). Amlodipine was a gift from Pfizer (Groton, CT).

Animals

Fischer 344 male rats derived from the National Institutes of Health colony were purchased from Harlan (Indianapolis, IN). Rats were used at 4, 13, and 23 mo of age (n = 6 for each group). After sacrifice, the left kidneys were removed, decapsulated, weighed, and used for measurement of O2 consumption. Cortex of right kidneys was snap-frozen in liquid N2 and stored at −80°C for immunoblotting studies. The protocols used conform to the Guiding Principles for the Care and Use of Laboratory Animals of the American Physiologic Society and the National Institutes of Health.

Preparation of Kidney Tissue Slices and Measurement of O2 Consumption

Thin slices of cortex (approximately 1 mm thickness; weight 10–20 mg) were prepared and incubated in Krebs bicarbonate solution (containing 118 mmol/L NaCl, 4.7 mmol/L KCl, 1.5 mmol/L CaCl2, 25 mmol/L NaHCO3, 1.2 mmol/L KH2PO4, 1.1 mmol/L MgSO4, and 5.6 mmol/L glucose; 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 HEPES 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 using an O2 monitor (model YSI 5300) connected to a linear chart recorder (model 1202; Barnstead/Thermolyne Corp, Dubuque, IA). Dose response curves of O2 consumption were expressed as a percentage change from baseline O2 consumption. Baseline O2 consumption was measured in the cortex in the absence and the presence of L-NAME (10−3 mol/L) in 4-, 13-, and 23-mo-old rats (n = 6 for each group).

Effect of Agonists of NO Production 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 (n = 6 for each group) to stimulate endogenous NO production. The response to these drugs was also examined after preincubation with the NOS inhibitor L-NAME (10−3 mol/L) to verify the role of NO production in the regulation of O2 consumption.

Effect of Superoxide Scavenging or Inhibition on O2 Consumption

To determine the role of superoxide radical production on NO availability and O2 consumption, the superoxide scavenger tempol (10−3 mol/L) was added before the addition of bradykinin, enalaprilat, and amlodipine in incubations with tissue from all three groups of rats (n = 6 each). The effect of inhibition of oxygen radical production on O2 consumption was assessed by addition of the NAD(P)H oxidase inhibitor apocynin (10−5 mol/L) to incubations with tissue from 23-mo-old rats (n = 6).

Effect of NO Donor on O2 Consumption

s-nitroso-N-acetylpenicillamine (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) and tempol (10−3 mol/L) (n = 6 each group).

Measurement of eNOS Protein Levels

Renal cortex was snap frozen in liquid nitrogen and stored at −80°C. For preparation of extracts, tissue was pulverized in liquid nitrogen followed by homogenization in 5 volumes of lysis buffer (0.05 M Tris-HCl, pH 7.2, 1 mM EDTA, 10 mM dithiothreitol, 1 mg/ml PMSF, 100 μg/ml leupeptin, 100 μg/ml soybean trypsin inhibitor, and 20 μg/ml aprotinin) at 4°C and sonication for 1 min. Lysates were centrifuged at 10,000 g for 10 min at 4°C and stored at −80°C before use. Protein content of supernatants was measured using Bio-Rad protein assay (Bio-Rad Laboratories).

Samples of tissue lysate (100 μg of protein) were loaded into individual lanes, subjected to electrophoresis on 10% polyacrylamide gels, and electrophoretically transferred from the gels to PVDF membranes (Amersham Pharmacia Biotech) using a semi-dry transfer cell (Bio-Rad). Membranes were blocked for 1 h with 5% milk/PBS and incubated with affinity-purified monoclonal antibody to eNOS (BD Transduction Laboratories) and β-actin (Novus Biologicals, Inc., Littleton, CO) in 1% milk/PBS at 4°C overnight. After incubation with horseradish peroxidase–conjugated rabbit anti-mouse IgG (Amersham Pharmacia Biotech), sites of antibody-antigen reaction were visualized using Super Signal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL) followed by exposure to x-ray film (Kodak, Rochester, NY).

The relative intensities of bands in autoradiograms were determined on an Alphalager 2000 documentation and analysis system (Alpha Innotech Corporation, San Leandro, CA) followed by analysis using image software.

Chemiluminescence Measurement of Superoxide

Production of superoxide by renal cortical tissue was assessed using lucigenin-enhanced chemiluminescence. All manipulations
were performed in a darkroom with minimal light. Plastic scintillation
minivials containing 10 μmol/L lucigenin and all agonists or inhibi-
tors in a final volume of 1 ml of air-equilibrated Krebs solution
buffered with 10 mmol/L HEPES (pH 7.4) were dark adapted for 5
min. These blanks were counted once, followed by addition of slices
of renal cortex (10 to 20 mg) and recounting three times. Renal
cortical tissue was preincubated in buffer containing the same com-
ponents as the scintillation vials before addition to the vials. The
chemiluminescence elicited by superoxide in the presence of lucige-
nin was measured in a liquid scintillation counter (Mark V, TmAna-
lytic) with a single active photomultiplier tube positioned in out-of-
coincidence mode. Data are reported as counts per minute (CPM) per
mg tissue (wet weight) after subtraction of the background.

Statistical Analyses
All data are expressed as mean ± SEM. Statistical analysis of
baseline O2 consumption was performed using t test. Changes in O2
consumption and eNOS protein levels were analyzed using ANOVA
followed by the Bonferroni correction for multiple comparisons (Sig-
maStat, SPSS-Science, Chicago, IL). Statistical significance was
achieved at P < 0.05.

Results

Baseline Renal Cortical O2 Consumption
Baseline renal cortical tissue O2 consumption was not dif-
f erent in the three age groups (4 mo: 692 ± 11 nmol O2/min/g,
n = 6; 13 mo: 704 ± 20 nmol O2/min/g, n = 6; 23 mo: 681 ±
29 nmol O2/min/g, n = 6, P > 0.05). Addition of the NOS
inhibitor L-NAME (10⁻³ mol/L) did not significantly alter O2
consumption in any group (4 mo: 720 ± 11 nmol O2/min/g, n
= 6; 13 mo: 735 ± 20 nmol O2/min/g, n = 6; 24 mo: 720 ±
27 nmol O2/min/g, n = 6, P > 0.05), suggesting that basal
levels of NO production, in the absence of blood flow, are not
significantly affecting O2 consumption.

Effect of Bradykinin on Renal O2 Consumption
Cumulative doses of bradykinin (10⁻³ to 10⁻⁴ mol/L) pro-
duced significant, dose-dependent decreases of renal cortical
O2 consumption in 4-, 13-, and 23-mo-old rats (4 mo: from
−2.5 ± 0.9% to −20.6 ± 1.5%, n = 6; 13 mo: from 0 ± 0% to
−17.7 ± 2.1% n = 6; 23 mo: from 0 ± 0% to −13.4 ±
0.9% n = 6) (Figure 1). Addition of L-NAME blocked the
effect of bradykinin, suggesting a dependence on NOS activity
(4 mo: from 0 ± 0% to −13.7 ± 3.3%, n = 6; 13 mo: from 0
± 0% to −11.2 ± 1.1%, n = 6; 23 mo: from 0 ± 0% to −7.6
± 1.3%, n = 6) (Figure 1, A through C). The suppression of
cortical O2 consumption by bradykinin was significantly less in
the 23-mo-old rats when compared with either group of
younger animals (Figure 1D). This suggests decreased NO
production in the older animals, which is further supported by
the reduced effect of L-NAME in 23-mo-old rats (Figure 1C).

Effect of Enalaprilat on Renal O2 Consumption
The angiotensin-converting enzyme (ACE) inhibitor enala-
prilat (10⁻⁷ to 10⁻⁴ mol/L), which stimulates endogenous NO
production, similarly caused concentration-dependent de-
creases in renal cortical O2 consumption in 4-, 13-, and 23-
mo-old rats (4 mo: from −0.7 ± 0.5% to −26.0 ± 1.2%, n
= 6; 13 mo: from 0 ± 0% to −21.6 ± 1.5%, n = 6; 23 mo: from
−0.3 ± 0.3% to −16.7 ± 2.1%, n = 6) (Figure 2, A through
C). This effect was again reversed by the addition of L-NAME in
the presence of 10⁻⁶ mol/L or greater enalaprilat (4 mo:

![Figure 1](image-url)

Figure 1. Effect of cumulative doses of bradykinin on renal cortical O2 consumption in (A) 4-mo (circles), (B) 13-mo (triangles), or (C) 23-mo-old (squares) Fischer 344 rats in the absence (closed symbols) or presence (open symbols) of L-NAME (10⁻³ mol/L). Each condition was tested in six animals. Bradykinin caused dose-dependent decreases in O2 consumption in all groups that were significantly reversed by L-NAME (*P < 0.05 versus stimulation in the absence of L-NAME). (D) The response of tissue from 23-mo-old rats was significantly less than that of tissue from 4- or 13-mo-old rats as indicated (*P < 0.05 versus 4- or 13-mo-old rats).
Effect of Amlodipine on Renal O₂ Consumption

Amlodipine (10⁻⁷ to 10⁻⁵ mol/L), which also stimulates NO production, decreased renal cortical O₂ consumption in 4-, 13-, and 23-mo-old rats (4 mo: from −1.3 ± 0.9% to −18.0 ± 1.2%, n = 6; 13 mo: from 0 ± 0% to −15.6 ± 1.5%, n = 6; 23 mo: from 0 ± 0% to −13.6 ± 2.0%, n = 6) (Figure 3, A through C). Addition of L-NAME significantly attenuated amlodipine-induced decreases in O₂ consumption (4 mo: from 0 ± 0% to −8.6 ± 2.3%, n = 6; 13 mo: from 0 ± 0% to −8.9 ± 2.1%, n = 6; 23 mo: from 0 ± 0% to −9.4 ± 2.0%, n = 6), demonstrating the importance of NO synthesis by NOS in the effect of amlodipine (Figure 3). The suppression of cortical O₂ consumption by amlodipine was lower in 23-mo-old rats compared with 4- and 13-mo-old rats (Figure 2C). This again suggests reduced NO availability in the oldest animals and is also supported by a smaller effect of L-NAME in the 23-mo-old rats (Figure 2C).

Effect of Superoxide Scavenging or Inhibition on Renal O₂ Consumption

Tempol, which functions as a superoxide radical scavenger, was added to assess the possible role of excess superoxide in decreasing NO availability. In the presence of tempol the ability of bradykinin, enalaprilat, and amlodipine to decrease renal oxygen consumption in 23-mo-old rats was restored to levels seen in 4- and 13-mo-old rats (Figure 4). This suggests destruction of NO by superoxide as a mechanism for the decreased responsiveness to bradykinin, enalaprilat, and amlodipine in the 23-mo-old tissue. A similar improvement in responsiveness to all three drugs was seen in renal cortex from 23-mo-old rats after the addition of apocynin, an inhibitor of NADPH oxidase (Figure 5), suggesting that the NADPH oxidase complex is the source of the increased superoxide production.

Effect of an NO Donor (SNAP) on Renal O₂ Consumption

Administration of cumulative doses of the NO donor SNAP (10⁻⁷ to 10⁻⁴ mol/L) reduced renal cortical O₂ consumption in the three groups of rats to a similar degree (4 mo: from 0.5 ± 0.5% to −43.1 ± 2.6%, n = 6; 13 mo: from 0 ± 0% to −46.0 ± 3.4%, n = 6; 23 mo: from 0 ± 0% to −42.3 ± 2.2%, n = 6; P > 0.05). Addition of L-NAME had no effect on the response to SNAP, suggesting no impairment in the ability of the tissue from any age rat to respond to NO. Addition of tempol (Figure 4D) or apocynin also had no significant effect on response to SNAP (data not shown).

eNOS Protein Levels

eNOS protein levels were assessed by immunoblotting of lysates of renal cortical tissue from 4-, 13-, and 23-mo-old rats and compared with levels of β-actin in the same samples. The absolute levels of band intensity for eNOS and the ratios of eNOS/β-actin band intensity were not different in the three age
groups (eNOS/β-actin density at 4 mo: 2.05 ± 0.56; 13 mo: 2.02 ± 0.38; 23 mo: 2.08 ± 0.36; \( P > 0.05 \)) (Figure 6).

**Superoxide Production by Renal Cortical Tissue**

Production of superoxide by renal cortical tissue was assessed using lucigenin-enhanced chemiluminescence (Figure 7). Superoxide release was significantly increased in renal cortical tissue from 23-mo-old rats when compared with tissue from 4-mo-old animals (82852 ± 12403 cpm/mg *versus* 47558 ± 6147 cpm/mg; \( P < 0.05 \)). Addition of tempol significantly decreased superoxide production in 23-mo-old rats to levels seen in 4-mo-old animals (44607 ± 5646 cpm/mg; \( P < 0.05 \), but it did not significantly alter production in 4-mo-old animals (28582 ± 6311 cpm/mg).

**Discussion**

Our studies demonstrate that the regulation of renal cortical oxygen consumption by stimulators of NO production is im-
paired in tissue from older animals, suggesting an impaired production of NO in the kidney with aging. Levels of eNOS protein, however, are not altered with age, suggesting that decreased NO bioactivity is not a result of loss of this enzyme. Treatment with tempol, a superoxide radical scavenger, and apocynin, an inhibitor of NAD(P)H oxidase, restored the effect of drugs that stimulate NO production on oxygen consumption, suggesting that NO is being scavenged by combination with superoxide and that the NAD(P)H oxidase complex is a major source of the superoxide. Results with lucigenin-enhanced chemiluminescence further substantiate enhanced superoxide production in the renal cortex of older animals. Responsiveness to the exogenous NO donor SNAP is not impaired in tissue from older rats, suggesting that the effect of NO on mitochondrial respiration is intact. Different sites of production and effect of NO when stimulated by agonists as opposed to exogenous administration may explain a lack of apparent effect of superoxide to scavenge SNAP-derived NO. Thus, agonist-stimulated NO production is probably predominantly in endothelial cells, where it is scavenged by superoxide before it is able to diffuse to adjacent interstitial and tubular cells, whereas exogenous NO can act directly at these sites and may have less exposure to superoxide.

Our previous work in eNOS-deficient mice suggested that NO produced by eNOS was the prime regulator of renal cortical oxygen consumption in the in vitro assay we have used in the studies presented here (10). Accordingly, it is important that we did not detect a change in eNOS protein levels in the renal cortex with aging. However, we cannot exclude a contribution of nNOS and iNOS derived NO in this mechanism in
the normal kidney, and certainly not in pathologic conditions where there may be changes in expression of nNOS and iNOS. Since we could not quantitate nNOS or iNOS in the renal cortex in these studies, we cannot comment on possible contributions by changes in these NOS isoforms to altered NO production in aging. However, the ability of tempol and apocynin to restore the response in older animals to levels similar to those of younger animals argues against an underlying change in NO production and supports the thesis that scavenging by oxygen radicals is decreasing NO availability.

Increased oxidant stress, leading to decreased NO bioavailability in the kidney, is being revealed as an important pathologic mechanism in several nephropathies. Defects in NO production and/or increases in oxidant stress, related to increases in production by NAD(P)H oxidase or decreases in radical scavenging systems, have now been demonstrated in diabetes, hypertension, aging, and chronic renal insufficiency. Superoxide rapidly combines with NO to form peroxynitrite, effectively limiting the availability of NO produced in the presence of superoxide and contributing to decreased NO bioavailability in the presence of increased oxygen radicals (16).

Renal NO production is frequently increased in early diabetics, although some studies have suggested decreased NO effect (reviewed in reference 17). For example, acetylcholine-induced relaxation of perfused rabbit afferent arterioles, an NO mediated effect, is decreased in arterioles from rabbits with STZ-induced diabetes, and this defect can be reversed by the addition of the superoxide radical scavenger tempol, suggesting oxidative stress as the cause of the decreased NO (18). Increased renal expression of a component of the NAD(P)H oxidase complex has been demonstrated in kidneys of STZ-induced diabetic rats, along with evidence of increased oxidant production as demonstrated by increased levels of \( \text{H}_2\text{O}_2 \), oxidatively modified lipids, and nitrosylated proteins (the product of peroxynitrite interaction with tyrosine residues) (19). Increased levels of oxidatively modified proteins in the renal cortex were also demonstrated in another study of STZ-induced diabetes, although nitrotyrosine levels were decreased, an effect felt to be due to overall decreased NO production in uncontrolled diabetes (20). We have found that in obese Zucker rats, a model of type II diabetes, inhibition of renal cortical oxygen consumption by bradykinin is also impaired and is restored both by superoxide scavenging with tempol and inhibition of NAD(P)H oxidase with apocynin (unpublished observations), again suggesting a role of enhanced superoxide production in decreased NO availability.

Enhanced oxidative stress is manifested in several models of hypertension, as well as in humans with essential hypertension (reviewed in reference 21). In the SHR, administration of tempol, a superoxide radical scavenger, normalizes BP and decreases evidence of oxidant stress (22,23). Prevention of the BP-lowering effect of tempol by infusion of \( \text{L}-\text{NAME} \), which inhibits NO synthesis, suggests that NO destruction by superoxide contributes to hypertension in SHR (22). Vaziri et al. (24) also found evidence of oxidant stress in young SHR with improvement of BP after antioxidant therapy. We have found abnormalities of intrarenal NO production in the SHR, a defect reversed by tempol, suggesting destruction of NO by superoxide (12). In lead-induced hypertension, there is evidence of enhanced tissue nitrotyrosine content along with decreased urinary excretion of NO metabolites, also suggesting NO destruction by superoxide (25). However, further study of these animals failed to show significant changes in renal NAD(P)H oxidase or antioxidant systems, leaving the source of superoxide radicals unclear (26). Our results with apocynin suggest that at least in aging, NAD(P)H oxidase may be an important source of intrarenal superoxide.

In humans with renovascular disease, oxidant stress is increased as manifested by enhanced urinary excretion of 8-iso-prostaglandin \( \text{F}_2\alpha \), a breakdown product of oxidatively modified prostaglandins (27). Basal superoxide release is detectable in arteries and veins from patients undergoing coronary artery bypass surgery, most of whom are hypertensive, and appears to occur throughout the vessel wall (28). Another group found that in vessels from similar patients, apocynin decreased superoxide generation, restored endothelium-dependent relaxation in an NO-dependent manner, and increased NO production from cultured human endothelial cells (29). Similar results were seen in vessels from stroke-prone SHR (29). While the vasculature has usually been assumed to be an important source of superoxide, in the model of hypertension induced by chronic NOS inhibition renal tubular epithelial cells appear to be an important site of oxidant stress (30).

In the 5/6 nephrectomy model of chronic renal insufficiency in the rat, there is evidence of increased vascular superoxide production contributing to hypertension and preventing acetylcholine-induced vascular relaxation (31). Another group found in similar animals that expression of the gp91phox subunit of NAD(P)H oxidase was increased while expression of two isoforms of superoxide dismutase (SOD), Cu/Zn SOD, and MnSOD were decreased, leading to decreased NO availability (32). In addition to decreasing NO availability, oxidant stress may promote renal fibrosis, leading to progressive renal failure (33). Oxidant stress in the kidney may also contribute to development of acute renal failure. In endotoxin-induced acute renal failure in mice, loss of the antioxidant effect of extracellular SOD appears to account for decreased NO availability and worsening of renal function (34).

Despite decreased urinary excretion of \( \text{NO}_x \) in aged male Sprague Dawley rats, the control of the renal vasculature by NO appears intact in these animals and NO may play a more important role in the maintenance of renal perfusion in older rats (5,6). However, more recent work from one of these laboratories has found decreased levels of NOS activity and eNOS and nNOS protein in similarly aged male rats (8). Decreased NO bioavailability has been reported in older Wistar-Kyoto (WKY) rats and stroke-prone SHR and appears to be related to increased superoxide production (35). In old SHR, decreased renal expression of eNOS and inducible NOS was found, although these changes may be secondary to hypertension-induced nephrosclerosis, and the decrease was prevented by chronic therapy with an angiotensin receptor blocker, which also prevented chronic renal injury (36). Our work supports decreased NO bioavailability in the aging renal
cortex, but as a result of increased destruction rather than decreased production or loss of eNOS enzyme. Differences between these studies may reflect species differences or differences in methodology.

Loss of NO availability in the kidney with aging would be expected to result in several adverse effects, including a decrease in renal perfusion, predisposition to the development of renal failure, and enhancement of fibrosis. Interference with NO synthesis leads to aggravation of hypoxic injury in the renal medulla in rats (14). NO may play a role in modulating matrix deposition in the kidney, and lack of NO may lead to fibrosis by increasing mesangial cell proliferation, collagen production, secretion of fibrogenic cytokines and interstitial fibrosis (37–42). Intrarenal pO2 may also be lowered by decreased NO availability and increased O2 consumption, as seen in the SHR (13), and this may contribute to renal fibrosis through activation of hypoxia-inducible genes (43,44). Loss of NO may also play a role in enhancing susceptibility to apoptosis in aging endothelial cells, a process reversed by exogenous NO or overexpression of eNOS (45). Loss of microvascular endothelium may then lead to progressive glomerular and interstitial scarring resulting in progressive renal disease (46). Thus loss of NO availability might contribute to the formation of the renal lesions seen in aging by several different mechanisms.

In summary, we have demonstrated decreased NO bioavailability in renal cortex of aging Fischer 344 rats. These changes appear to be due to scavenging of NO by increased superoxide generation from NAD(P)H oxidase and are reversed by an antagonist of NAD(P)H oxidase or a scavenger of superoxide. Alterations in renal cortical eNOS levels do not appear to play a role in these changes. Whether augmentation of NO production or decreases in renal oxidant stress can preserve renal function in aging is worthy of study.

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References


