Vitamin E Alleviates Renal Injury, but Not Hypertension, during Chronic Nitric Oxide Synthase Inhibition in Rats

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Abstract. Chronic nitric oxide (NO) synthase (NOS) inhibition in rats causes hypertension, renal vascular injury, and proteinuria. NO deficiency increases superoxide (O$_2^-$) activity, but the effects of antioxidant treatment on renal injury have not been studied in this model. Exposure of rats to Nω-nitro-L-arginine (L-NNA) for 4 d markedly decreased NO-dependent relaxation in aortic rings and increased glomerular and renal interstitial monocyte influx, but renal O$_2^-$ activity was not increased. After 7 d, BP and proteinuria were significantly increased. After 21 d of L-NNA treatment, rats displayed severe hypertension, decreased GFR, marked proteinuria, glomerular ischemia, renal vascular and tubulointerstitial injury, and complete loss of NO-dependent relaxation. Renal O$_2^-$ activity was markedly increased [lucigenin-enhanced chemiluminescence (LEC), 279 ± 71 versus 50 ± 7 counts/10 mg, P < 0.01; electron paramagnetic resonance spectroscopy, 0.57 ± 0.05 versus 0.34 ± 0.04 U/10 mg, P < 0.05]. Apocynin, a specific inhibitor of NADPH oxidase, and diphenyleneiodonium, an inhibitor of flavin-containing enzymes, completely inhibited LEC signals in vitro, whereas allopurinol had no effect, indicating that NAD(P)H oxidase plays a major role in superoxide production in the kidney. Endothelial function remained impaired during cotreatment with α-tocopherol and there was no effect on hypertension or tubulointerstitial injury, but glomerular ischemia, decreases in GFR, and renal vascular injury were prevented and proteinuria was ameliorated. Renal LEC signals were intermediate between control and L-NNA-alone values (181 ± 84 counts/10 mg). Chronic NO synthase inhibition in rats results in marked increases in renal cortical O$_2^-$ activity, mediated by flavin-dependent oxidases. The absence of early increases in renal O$_2^-$ activity, in the presence of endothelial dysfunction and macrophage influx, indicates that increased renal O$_2^-$ activity is neither attributable to NO deficiency per se nor solely related to macrophage influx. The improvement of glomerular function and amelioration of renal vasculitis and proteinuria with vitamin E cotreatment indicate that oxidants are involved in the pathogenesis of renal injury in this model. However, markedly impaired endothelial function and unabated hypertension persist with vitamin E treatment and seem to be directly attributable to NO deficiency.

Chronic nitric oxide (NO) synthase (NOS) inhibition in rats results in hypertension, renal vascular injury, and proteinuria (1). Oxygen radicals, such as O$_2^-$, seem to be involved in pathophysiological processes in diverse models of chronic renal disease; deficiencies of the antioxidants vitamin E and selenium induced renal injury in intact rat kidneys (2), and vitamin E supplementation reduced glomerulosclerosis in the remnant kidney model (3). Tungsten, an inhibitor of xanthine oxidase, markedly reduced proteinuria in passive Heymann nephritis and aminonucleoside-induced nephrosis (4). However, whether O$_2^-$ plays a role in the pathogenesis of hypertension and renal injury induced by chronic NO deficiency is unknown.

Several mechanisms may contribute to increased O$_2^-$ levels during chronic NOS inhibition. First, NO can scavenge O$_2^-$ in a rapid reaction that yields peroxynitrite. It is unclear to what extent and in which compartments this occurs in vivo (5). Second, NO inhibits O$_2^-$ production via a direct action on NADPH oxidase (6). Acute NOS inhibition increased oxidative stress in the microvasculature in vivo (7). Third, we observed that all of the effects of chronic NOS inhibition could be prevented by AT$_1$ receptor blockade, suggesting an important role for angiotensin II (8). Angiotensin II stimulates O$_2^-$ production by NAD(P)H oxidase in vascular (9) and renal (10) cells. Recently, angiotensin II-induced hypertension was demonstrated to be dependent on vascular O$_2^-$ activity (9). Therefore, O$_2^-$ production may also contribute to the hypertension observed in this model. Increased aortic O$_2^-$ activity after chronic NOS inhibition was attenuated by removal of the endothelium after 1 wk (11) but not after 5 wk (12), suggesting that NAD(P)H oxidase in smooth muscle cells may also enhance vascular O$_2^-$ production when NO deficiency is pro-
longed. Therefore, NOS inhibition may increase $O_2^-$ production directly or $O_2^-$ production may be increased during injury. The latter may also exacerbate the injury. Finally, inflammation induced by chronic NOS inhibition is characterized by infiltration of macrophages into the renal cortex (13). Macrophages produce large amounts of $O_2^-$ (14). Therefore, during chronic NOS inhibition, there are direct effects of NO deficiency and indirect effects of injury that may increase renal $O_2^-$ activity.

In this study, we determined whether NO deficiency, either directly or secondarily to renal injury, results in increased renal cortical $O_2^-$ activity. Renal $O_2^-$ activity was measured after 4 d of NOS inhibition (when no renal injury is present) and after 21 d of NOS inhibition (when renal injury is extensive) (8). The second aim was to determine whether oxidants have a pathogenic role in the hypertension and renal damage associated with chronic NOS inhibition. For this purpose, rats were treated with $N\text{-}n\text{itro}-l\text{-}arginine (l-NNA) and a high dose of the lipid antioxidant vitamin E.

Materials and Methods

Animals

Male Sprague Dawley rats (125 to 150 g; Harlan-Olac, Blackthorn, UK) received a standard diet containing 82 mg/kg vitamin E (RMHTM; Hope Farms, Woerden, Netherlands) and had free access to tap water and chow. The protocol was approved by the Utrecht University Board for Studies in Experimental Animals. Four groups of rats were studied ($n = 8$ to 12). Control animals received tap water and standard chow. The second group received l-NNA (Sigma Chemical Co., St. Louis, MO), dissolved in drinking water (500 mg/L), for 4 d. The third group received l-NNA, dissolved in drinking water, for 21 d. The fourth group received l-NNA, dissolved in drinking water, and (+)-$\alpha\text{-tocopherol acetate (vitamin E; Sigma), mixed with finely ground chow (0.7 g vitamin E/kg body wt per d, for 21 d. Rats received fresh chow every day. Once each week, the rats were placed in metabolism cages for 24 h, with free access to water and food (supplemented with l-NNA and vitamin E for the respective groups). Urinary protein levels were determined with Coomassie Blue. Systolic BP (SBP) was measured in conscious rats by using a tail cuff (ITTC, San Diego, CA). Plasma and urinary creatinine concentrations were determined colorimetrically (Kit 555-A, Sigma) at the end of the experiment. Creatinine clearance was used as an estimate of GFR. One kidney was perfused for measurements of $O_2^-$ activity. The other was processed for histologic examinations. The thoracic aorta was removed, cleaned, and cut into rings for measurement of aortic $O_2^-$ activity and NO-dependent relaxation.

Lucigenin-Enhanced Chemiluminescence in Intact Renal Cortex

Lucigenin-enhanced chemiluminescence (LEC) has often been used to detect $O_2^-$ in vascular tissue (9,11,12). In this study, we validated LEC as a probe for $O_2^-$ activity in rat renal cortex, under control conditions and during chronic NOS inhibition, by comparing the data obtained in LEC assays with those obtained by using electron paramagnetic resonance (EPR) spectroscopy, a validated method for determination of $O_2^-$ activity (15). Rats were anesthetized with 60 mg/kg pentobarbital, administered intraperitoneally. Their kidneys were perfused with ice-cold carbonized Krebs-Hepes buffer. Perfusion and storage of tissue in an ice-cold buffer greatly improved LEC signal reproducibility. Because perfusion may also affect $O_2^-$ production (16), all kidneys were perfused for 3 min while perfusion pressure was kept constant at 120 mmHg. For measurement of renal cortical $O_2^-$ activity, the kidneys were decapsulated and four cortical slices (approximately $5 \times 5 \times 2$ mm) were cut with a tissue-slicing device. The remaining renal cortex was processed for measurement of $O_2^-$ activity by using EPR spectroscopy. The thoracic aorta was removed, placed in ice-cold buffer, cleaned of blood clots and periarterial tissue, and cut into rings (approximately 7 mm long).

$O_2^-$ activity was measured by using a LUMAT LB 9507 luminometer (Berthold, Wildbad, Germany). Tissue samples were preincubated for 5 min in 300 $\mu$L of buffer at room temperature. Background LEC was measured for 5 min. Three hundred microliters of lucigenin (bis-N-methyl acridinium nitrate; Sigma) (17) were injected, and LEC was measured for 20 min (sample rate, 1 Hz). The samples were dried overnight and weighed. LEC values were calculated as the average counts measured during the last 5 min minus the average background activity and were expressed as counts per 10 mg dry weight.

The signals obtained in LEC assays do not always originate from $O_2^-\text{; non-superoxide-derived LEC signals were observed for phospholipids and saphenous veins (18). Native superoxide dismutase (SOD) is not useful for assessment of specificity, because it scavenges only extracellular $O_2^-$\text{, whereas lucigenin reacts with extra- and intracellular $O_2^-$ (19). Tiron (4,5-dihydroxy-1,3-benzenesulfinic acid) is often used as an alternative for native SOD (10,14). Tiron enters cells readily but also acidifies tissue, which can affect both $O_2^-$ activity and LEC signals (17). Therefore, to determine LEC specificity for $O_2^-$, we added manganese(III)tetrakis(4-benzoic acid)porphyrin (MnTBAP) (300 $\mu$M; Alexis Biochemicals, San Diego, CA), a pH-neutral, membrane-permeable compound with SOD-like activity (20), or MnCl$_2$ (2 mM), another pH-neutral compound with SOD-like activity (21), after 20 min. MnCl$_2$ was used because in solution it has a light pink color, whereas the MnTBAP solution has a black color, which might absorb the luminescence signal. MnTBAP and MnCl$_2$ completely abolished the LEC signal induced by the combination of xanthine and lucigenin (80 $\mu$M; Sigma) and xanthine oxidase (50 $\mu$M; ICN, Costa Mesa, CA). It was demonstrated, in in vitro enzymatic systems that produced little $O_2^-\text{, that lucigenin itself can act as a source of $O_2^-$, via auto-oxidation of the lucigenin cation radical (22). Dose-response curves for lucigenin (3, 10, 100, and 300 $\mu$M) were obtained in renal tissue from control rats and rats treated with l-NNA, for determination of a concentration of lucigenin at which auto-oxidation does not occur (23). This concentration was 100 $\mu$M (see Results). The source of the renal $O_2^-$ activity was determined with the addition of 50 $\mu$M of allopurinol (Sigma), an inhibitor of xanthine oxidase, dissolved in buffer (1 mM) or 3 $\mu$M of diphenyleneiodonium chloride (DPI) (Sigma), an inhibitor of flavin-dependent oxidases, dissolved in DMSO (50 $\mu$M). Addition of DMSO at that concentration (3:600, vol/vol) did not affect LEC. LEC signals induced by the combination of xanthine and xanthine oxidase were abolished by allopurinol but were not affected by DPI.

Additional experiments were performed to identify the enzymatic source of $O_2^-\text{ in the renal cortex of rats treated with l-NNA for 3 wk (n = 10). Cortical slices were incubated in Krebs-Hepes buffer at room temperature for 30 min, without or with apocyinin, a specific inhibitor of the assembly of components of NADPH oxidase (100 $\mu$M; Aldrich, Milwaukee, WI). Subsequently, the samples were rinsed with buffer and LEC was determined as described.
Renal cortex was incubated with the lipid-soluble agent 2-N-t-butyl-\(\alpha\)-phenyl-nitronate (20 mM; Molecular Probes Europe BV, Leiden, The Netherlands) for 10 min, extracted with chloroform/methanol/demineralized water, dried overnight, and then weighed. Before measurements, the extract was reconstituted with chloroform. The chloroform was dried with anhydrous Na\(\text{2}\)SO\(\text{4}\) and deoxygenated by bubbling with N\(\text{2}\) (15). EPR spectroscopic experiments were performed with a Bruker ESP 300 X-band (approximately 9.5 GHz) spectrometer operating in TE\(_{102}\) cavity. The sample (200 \(\text{\mu\ell}\)) was drawn into a quartz capillary tube and placed within a rectangular cavity. Measurements were performed at room temperature, at 10 mW and 9.44 GHz. The spectrum of PBN was recorded with a magnetic field of 3358 G and a sweep width of 50 G, modulated at 100 kHz. The detector gain was \(6.3\times10^5\), the time constant was 655 ms, and the analog/digital conversion time was 82 ms. Accumulation of four scans enhanced the signal/noise ratio. EPR spectroscopic findings, calculated as the signal/noise ratio, were expressed as units per 10 mg dry weight.

Histologic and Immunohistochemical Analyses

Kidney slices were either immersion-fixed in phosphate-buffered formaldehyde (4%, pH 7.35) and embedded in paraffin or immersion-fixed in methacarn (60% methanol, 30% chloroform, and 10% acetic acid) for 24 h, stored in 70% ethanol, and subsequently embedded in paraffin. Light microscopy was performed on 3- \(\mu\text{m}\) sections of formaldehyde-fixed tissue stained with periodic acid-Schiff stain. Immunohistochemical analyses were performed on 5- \(\mu\text{m}\) sections of methacarn-fixed tissue, by using the alkaline phosphatase/anti-alkaline phosphatase technique (Dako, Hamburg, Germany). Tissue sections were incubated with the mouse monoclonal antibody ED-1 (Serotec/Camon, Wiesbaden, Germany), to demonstrate monocytes/macrophages, as described (13). One hundred glomeruli were evaluated for the presence of ischemia. ED-1-positive cells (monocytes/macrophages) were counted in all arteries, in 50 glomeruli, and in the tubulointerstitium (20 fields at \(\times400\) magnification). For each structure, an average score per rat was calculated. ED-1-positive cells in arteries were counted if they were attached to the endothelium or infiltrated into the intima or media. The pathologist was blinded to the groups.

NO-Dependent Relaxation

Aortic rings (approximately 4 mm) were maintained on ice in carbogenized Krebs buffer. Indomethacin (10 \(\mu\text{M}\)) was added to prevent the formation of prostaglandins. Rings were mounted in chambers filled with Krebs buffer (37°C), for measurement of isometric tension. After precontraction with phenylephrine (0.3 \(\mu\text{M}\)), dose-response curves were obtained with acetylcholine (1 nM to 0.1 mM). Acetylcholine-induced relaxation was completely blocked when 1-NNA was added, demonstrating that relaxation induced by acetylcholine was NO-dependent. Finally, nitroprusside (10 \(\mu\text{M}\)) was added for determination of smooth muscle integrity.

Statistical Analyses

Results are expressed as mean ± SEM. SBP and urinary protein excretion data were analyzed by two-way ANOVA for repeated measures, followed by the Student-Newman-Keuls test. Terminal data were analyzed by one-way ANOVA, followed by the Student-Newman-Keuls test for functional data and the Kruskal-Wallis test for morphologic data.
± 4 mg/d for control animals (*P < 0.05*), and GFR was significantly decreased (1.58 ± 0.14 ml/min *versus* 2.37 ± 0.15 ml/min for control animals, *P < 0.05*). Cotreatment with L-NNa and vitamin E delayed the development of proteinuria (Figure 2A) but did not affect SBP (Figure 2B). After 21 d of cotreatment, proteinuria (185 ± 29 mg/d) was significantly increased in comparison with control values (*P < 0.05*) but was significantly decreased in comparison with values for L-NNa alone (*P < 0.05*). GFR was 2.30 ± 0.38 ml/min, which was not significantly different from control values.

**O$_2^-$ Activity in the Renal Cortex and Aorta**

After 4 d of L-NNa treatment, renal cortical O$_2^-$ activity, as measured in LEC assays (40 ± 8 counts/10 mg), was not significantly different from control values (50 ± 7 counts/10 mg). Treatment with L-NNa for 21 d resulted in a marked increase in renal cortical O$_2^-$ activity (279 ± 71 counts/10 mg, *P < 0.05*). Cotreatment with vitamin E resulted in renal cortical O$_2^-$ activity (181 ± 84 counts/10 mg) that was intermediate between control and L-NNa-alone values. Similarly, 21 d of NOS inhibition increased O$_2^-$ activity in the aorta (100 ± 32 counts/10 mg *versus* 27 ± 4 counts/10 mg for control animals; *P < 0.05*); after cotreatment with vitamin E, aortic O$_2^-$ activity (47 ± 10 counts/10 mg) was intermediate. Increased O$_2^-$ activity seemed to be attributable to flavin-dependent oxidases, because addition of DPI reduced the renal cortical LEC signal to 13 ± 2 counts/10 mg in rats treated for 21 d with L-NNa and to 17 ± 4 counts/10 mg in rats treated with L-NNa plus vitamin E, whereas addition of allopurinol had no effect in either group. Addition of L-NNa (100 mM) *in vitro* did not reduce the LEC signal, suggesting that O$_2^-$ activity in the kidney is not derived from NOS. DPI, but not allopurinol or L-NNa, also reduced O$_2^-$ activity in the aorta.

In additional experiments with rats treated with L-NNa for 21 d (*n* = 10), which demonstrated similarly increased proteinuria and BP, cortical slices were incubated for 30 min at room temperature in Krebs-Hepes buffer without or with apocynin, a specific inhibitor of the assembly of NADPH oxidase components. These experiments clearly indicated that NAD(P)H oxidase is an important source of O$_2^-$ activity, because apocynin reduced the LEC signal from 134 ± 18 to 4 ± 1 counts/10 mg for rats treated for 21 d with L-NNa.

**Glomerular Ischemia and Renal Monocyte/Macrophage Influx**

Practically no glomerular ischemia was observed after 4 d of L-NNa treatment (Figure 3B and Table 1). Three weeks of

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**Table 1.** Renal glomerular ischemia and arterial, glomerular, and interstitial monocytes/macrophages in control rats and rats treated with L-NNa for 4 or 21 d or with L-NNa plus vitamin E for 21 d

<table>
<thead>
<tr>
<th>Treatment (d)</th>
<th>Control</th>
<th>L-NNa</th>
<th>L-NNa</th>
<th>L-NNa + Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular ischemia (%)</td>
<td>0 (0 to 0)</td>
<td>4</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Monocytes/macrophages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>arteries (no./artery)</td>
<td>0.27 (0 to 0.64)</td>
<td>0.21 (0.10 to 0.44)</td>
<td>1.07 (0 to 2.25)$^d$</td>
<td>0.28 (0 to 0.50)$^c$</td>
</tr>
<tr>
<td>glomeruli (no./glomerulus)</td>
<td>0.80 (0.2 to 1.18)</td>
<td>2.05 (1.66 to 2.66)$^b$</td>
<td>1.64 (1.12 to 2.00)$^d$</td>
<td>0.86 (0.04 to 2.90)$^c$</td>
</tr>
<tr>
<td>interstitium (no./field)</td>
<td>6.0 (3.4 to 9.3)</td>
<td>19.9 (15.9 to 24.3)$^b$</td>
<td>16.9 (13.9 to 35.5)$^b$</td>
<td>15.6 (1.8 to 24.7)$^d$</td>
</tr>
</tbody>
</table>

$a$ Values are median and range. L-NNa, Nω-nitro-L-arginine.

$b$ *P < 0.01* *versus* control.

$^c$ *P < 0.05* *versus* L-NNa (21 d).

$^d$ *P < 0.05* *versus* control.
1-NNA treatment produced ischemic signs (partial or complete tuft collapse) in 20% (range, 10 to 26%) of glomeruli ($P < 0.01$) (Figure 3C). This value was reduced to 4% (0 to 12%) after cotreatment with vitamin E ($P < 0.05$ versus 1-NNA alone) (Figure 3D). No increase in the number of ED-1-positive cells (monocytes/macrophages) was observed in arteries after 4 d of 1-NNA treatment, but the numbers of these cells were increased in glomeruli and interstitium ($P < 0.05$). 1-NNA treatment for 21 d resulted in vascular injury (Figure 3C) characterized by an increased number of ED-1-positive cells attached to the endothelium and infiltrated into the intima and media of arteries, in comparison with control samples ($P < 0.05$) (Table 1). The numbers of ED-1-positive cells in glomeruli and tubulointerstitium were also significantly increased.
Before discussing the role of $O_2^-$ in the pathogenesis of renal injury during chronic NOS inhibition, we must briefly comment on the use of LEC assays. The debate regarding the validity of LEC assays (18,22) can be reduced to the following two important questions: does the LEC signal originate from $O_2^-$ and does the LEC signal reflect $O_2^-$ derived from the biologic material? To test whether the LEC signal originated from $O_2^-$, we added the pH-neutral, membrane-permeable, SOD-mimetics MnTBAP (20) and MnCl$_2$ (21) to our samples. Both compounds immediately abolished the LEC signal, demonstrating that this signal reflects biologic $O_2^-$ activity. To examine whether the LEC signal could be attributable to auto-oxidation of the lucigenin cation radical (22), we compared the LEC signal with $O_2^-$ measurements obtained using EPR spectroscopy. Both types of measurements indicated increased $O_2^-$ activity after chronic 1-NNa treatment. Furthermore, lucigenin concentrations of 100 and 300 $\mu$M resulted in comparable signals, showing that, within this range, lucigenin is not auto-oxidized to a significant extent. The tendency of lucigenin to undergo redox cycling is apparently very limited in systems that produce significant amounts of $O_2^-$ (23). The findings that the signal obtained in LEC assays reflected $O_2^-$ activity and that the risk of auto-oxidation was small allow the use of this method for assessment of $O_2^-$ activity in renal tissue.

This study demonstrates for the first time that chronic NOS inhibition in rats for 21 d results in marked increases in renal cortical $O_2^-$ activity. Interestingly, after 4 d of NOS inhibition, renal cortical $O_2^-$ activity was not different from control values. This suggests that the increased renal cortical $O_2^-$ activity is not a direct effect of NOS inhibition but is secondary to renal injury. This was supported by the observation that the LEC signal was not reduced by the addition of 1-NNa in vitro. The increased $O_2^-$ activity probably resulted from flavin-dependent oxidases, because addition of DPI could completely inhibit the LEC signal. Several $O_2^-$-generating systems using flavin-dependent oxidases may be activated by renal injury. Angiotensin II stimulates $O_2^-$ production by the flavin-dependent enzyme NAD(P)H oxidase in the vasculature (9) and in kidneys (10). We previously observed complete prevention of all effects of chronic NOS inhibition with AT$_1$ receptor blockade (8). In addition, chronic NOS inhibition is characterized by severe proteinuria and increased macrophage infiltration into the tubulointerstitium (13). In proteinuric states, iron-containing proteins such as transferrin are delivered to renal tubules. Heme iron is essential for $O_2^-$ generation by membrane-bound NADPH oxidase (24). Because the specificity of DPI as an inhibitor of NAD(P)H oxidase has recently been challenged (25), we incubated samples of renal cortex with apocynin, a specific inhibitor of the assembly of the components of NADPH oxidase (26,27). Incubation with apocynin completely inhibited the LEC signal. To our knowledge, this is the first report of the use of apocynin to demonstrate that NAD(P)H oxidase plays a major role in superoxide production in the kidney. The inhibitory effects of apocynin on the assembly of NAD(P)H oxidase have been demonstrated in endothelial cells (28), vascular smooth muscle cells (29), and macrophages (30). Considering the extent of injury after 21 d of 1-NNa treatment,

![Graph](image)

**Figure 4.** Nitric oxide-dependent relaxation of isolated aortic rings from control rats (●) and rats treated for 4 d with l-NNa (▲), for 3 wk with l-NNa (■), or for 3 wk with l-NNa plus vitamin E (▲).
it is likely that multiple systems using NADPH oxidase were involved.

For study of the role of oxidants in the pathogenesis of renal injury and vascular dysfunction induced by chronic NOS inhibition, rats were treated with a high dose of the lipid-soluble antioxidant vitamin E. Vitamin E was chosen because it is the major antioxidant in lipid bilayers (2). Recent evidence suggests that vitamin E can be used to reduce cardiovascular disease among patients with end-stage renal disease (31). It should be noted that, in addition to its direct antioxidant effects, α-tocopherol can provide some protection by affecting signaling functions in smooth muscle cells (32). At levels lower than those used in this study, vitamin E administration increased plasma (33) and renal cortical (34) vitamin E levels and reduced renal injury in diverse models of chronic renal disease, such as the remnant kidney model (3,33) and diabetes mellitus (34). Indeed, vitamin E reduced renal cortical O2 activity in parallel with amelioration of l-NNA-induced renal vascultis and glomerular ischemia, resulting in full recovery of the GFR. Because hypertension, vascultis, and glomerular ischemia in this model of chronic NOS inhibition were all prevented by losartan (8) and because SOD can reduce angiotensin II-induced hypertension (9), it is remarkable that vitamin E did not relieve the hypertension induced by chronic NOS inhibition. In fact, BP was numerically even higher in the vitamin E-treated group (Figure 2B). This points to a direct effect of NO availability on BP control, possibly via medullary NO (35). It has been demonstrated that antioxidants reduce hypertension in spontaneously hypertensive rats (36). However, spontaneously hypertensive rats exhibit increased, rather than decreased, renal and vascular NOS activity (e.g., reference 37). Inactivation of NO is apparently increased secondary to oxidative stress in spontaneously hypertensive rats, whereas oxidative stress is secondary to decreased NO availability in our model. Indeed, three different antioxidants (allopurinol, Ebselen [a selenium compound that mimics glutathione peroxidase], and N-acetylcysteine) did not restore aortic NO production and had no antihypertensive effect in rats treated with N\(^2\)-nitro-L-arginine methyl ester for 7 d (11). Although in our study vitamin E halved aortic O\(_2^−\) activity, NO-dependent vascular relaxation in the aorta was improved only approximately 10%. In contrast, endothelium-dependent relaxation was fully restored by heparin-binding SOD in renovascular hypertension (38) and was restored approximately 50% by vitamin E in hypercholesterolemia (39). However, those models are characterized by inactivation of NO secondary to oxidative stress, whereas oxidative stress is secondary to decreased NO availability in our study. Therefore, decreased NO availability, and not oxidative stress, seems to be responsible for decreased endothelium-dependent relaxation in the aorta and hypertension after 4 to 7 d of chronic NOS inhibition. In agreement with this are the observations that there was no early increase in BP in the vitamin E-deficiency renal injury model (2) and that vitamin E ameliorated renal injury in the remnant kidney model without decreasing BP (3).

Can oxygen radicals be held responsible for the proteinuria associated with chronic NOS inhibition? Dismutation of increased O\(_2^−\) by endogenous SOD results in increased H\(_2\)O\(_2\) generation. It was demonstrated that, when H\(_2\)O\(_2\) was infused into the renal artery, a 50-fold increase in urinary protein excretion occurred, without any change in arterial pressure (40). In our study, treatment with vitamin E reduced O\(_2^−\) levels and thus (by reducing the levels of the substrate for SOD) H\(_2\)O\(_2\) formation. This may have contributed to the significant retardation of the development of proteinuria induced by chronic NOS inhibition. Nevertheless, proteinuria remained significantly increased, compared with control values. The unabated hypertension probably also contributed to glomerular protein leakage. Although pathologic changes develop very rapidly in this model and such changes are probably not uniformly distributed throughout each target organ, establishment of a plausible chain of events from our findings is possible. An NO deficiency would directly decrease endothelial dilation, increase monocyte adhesion, and begin to increase BP, although we could not detect the latter change by external measurements before 7 d. Loss of glomerular permeability (resulting in protein leakage), in combination with localized loss of endothelial barrier function, could contribute to early renal interstitial monocyte influx. Persistent hypertension and unbalanced angiotensin II action stimulate NAD(P)H oxidase in vascular smooth muscle to produce O\(_2^−\). This has major consequences for glomerular integrity, leading to loss of glomerular hydraulic conductivity and permeability and resulting in manifest proteinuria. The latter, in turn, increase tubulointerstitial damage, monocyte influx, and oxygen radical production. At this dose of l-NNA, all rats die within 1 to 2 mo, probably as a result of renal failure, although death can sometimes be caused by cardiac (41) or spinal cord (42) infarctions.

Chronic NOS inhibition resulted in striking increases in renal cortical and aortic O\(_2^−\) activity. Scavenging of oxygen radicals and prevention of lipid oxidation with vitamin E maintained renal vascular and glomerular function and structural integrity. These beneficial effects were achieved despite persistent NO deficiency, as manifested by hypertension and decreased aortic endothelium-dependent relaxation.

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References


