Nitric Oxide Blockade Enhances Renal Responses to Superoxide Dismutase Inhibition in Dogs

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Abstract—To examine the potential role of superoxide anion (O$_2^-$) and its interaction with NO in the regulation of renal hemodynamics and excretory function, we have evaluated the renal responses to enhancement in O$_2^-$ activity before and during NO synthase inhibition in anesthetized dogs (n = 6). Intraarterial infusion of a superoxide dismutase (SOD) inhibitor, diethyldithiocarbamate (DETC; 0.1 and 0.5 mg/kg per min) was made to enhance O$_2^-$ activity in the kidney. Cortical (CBF), medullary (MBF), and total renal blood flow (RBF) responses were assessed using laser-Doppler needle flow probes and an electromagnetic flow probe. DETC caused dose-dependent changes in renal parameters, which were recovered within 30 minutes after the termination of DETC infusion. The high-dose infusion of DETC for 25 minutes resulted in an increase of 29±10% in renal vascular resistance (control, 35.4±4.4 mm Hg/mL per min per g) and decreases of 21±5% in RBF (control, 3.5±0.5 mL/min per g), 20±5% in CBF, 21±7% in MBF, 62±11% in urine flow (control, 10.5±2.2 μL/min per g), and 47±11% in sodium excretion (control, 2.1±0.2 μmol/min per g), without a significant change (−10±6%) in glomerular filtration rate (control, 0.74±0.09 mL/min per g). During NO synthase inhibition with intraarterial administration of nitro-L-arginine (50 μg/kg per min), the same dose of DETC showed a greater increase in renal vascular resistance (73±15%) and reductions in RBF (39±4%), CBF (32±5%), MBF (34±6%), urine flow (78±5%), and sodium excretion (67±10%), with a marked reduction in glomerular filtration rate (59±7%). These data indicate that O$_2^-$ exerts renal vasoconstriction as well as antidiuretic and antinatriuretic effects. These responses are enhanced during NO synthase blockade, suggesting that NO serves a renoprotective effect against these action of O$_2^-$. (Hypertension. 2002;39:293-297.)

Key Words: renal hemodynamics □ renal regional blood flow □ sodium excretion □ diethyldithiocarbamate

Partial reduction of molecular oxygen in living tissues can generate reactive oxygen species (ROS), including hydrogen peroxide and the free radicals superoxide (O$_2^-$) and hydroxyl ions. Over the past decade, there has been accumulating evidence for a role of ROS in the pathogenesis of a variety of renal diseases that involve vascular, glomerular, tubular, and renal interstitial damage. Recent studies have indicated a strong association of hypertension with the injurious effects of O$_2^-$, which is a relatively abundant formation in the living tissue. Both short-term and long-term inhibition of endogenous O$_2^-$ formation have been shown to reduce blood pressure in spontaneously hypertensive rats.

Although the role of O$_2^-$ in hypertension has been suggested in many studies, the exact mechanism involved in this pathophysiology is not yet clearly established. It is known that O$_2^-$ reacts with NO to form peroxynitrite, which could oxidize arachidonic acid and release a potent vasoconstrictor substance, 8-isoprostane. The reaction between NO and O$_2^-$ to form peroxynitrite has a 3-fold faster rate constant than that of the reaction of superoxide dismutase (SOD) enzyme with O$_2^-$ to form hydrogen peroxide. It has been suggested that the interaction of NO with O$_2^-$ has greatly influence in determining the extent of vascular reactivity to NO in the biological tissue. Under normal conditions, O$_2^-$ is a minor but constant product of the cellular metabolism. As the presence of O$_2^-$ can diminish the half-life of NO, the potential role of O$_2^-$/NO interaction in the regulation of many biological events has been a major focus of many recent studies. It has been suggested that NO plays an important cytoprotective role against the injurious effects of O$_2^-$ by acting as an antioxidative agent. Although experimental evidence has been amassed to indicate a key mediatory role for O$_2^-$ in the pathophysiological processes of various renal disease entities, its possible role in the physiological regulation of renal function is not yet clear in the literature. Recent studies in rats by Zou et al indicated that O$_2^-$ production in the renal medulla exerts vasoconstrictor as well as antidiuretic and antinatriuretic actions in the kidney. However, the role of O$_2^-$ in regulating whole kidney hemodynamics, particularly in the control of glomerular filtration rate (GFR), has not been addressed in...
any previous study. Moreover, an interactive role of O$_2^-$ and NO in the regulation of whole kidney blood flow and excretory function is not yet clearly defined.

The present investigation was designed to examine the hypothesis that NO activity reciprocally regulates intrarenal O$_2^-$ level, which has direct influence on renal hemodynamics and tubular function, leading to alterations in net sodium reabsorption rate. The objective of this study is to evaluate the effects of enhanced O$_2^-$ level and its interaction with NO in the control renal hemodynamics and excretory function. In these experiments, renal responses to SOD inhibition with diethyldithiocarbamate (DETC) were assessed before and during NO inhibition with nitro-$l$-arginine (NLA)$^{16-18}$ in anesthetized dogs.

**Methods**

The experiments were performed in accordance with the guidelines and practices established by the Tulane University Animal Care and Use Committee.

Experiments were performed in 6 mongrel dogs (17 to 22 kg body wt) of either gender. To achieve a sodium replete state, the dogs were anesthetized with pentobarbital sodium (30 mg/kg IV) and given supplemental amounts of sodium chloride (1.5 g/kg body wt) of either gender. To achieve a sodium replete state, the dogs were given supplemental amounts of sodium chloride (1.5 g/kg body wt) added to the normal laboratory diet. The animals were anesthetized with pentobarbital sodium (30 mg/kg IV) and given additional doses as required. The surgical preparation of the animals and basic experimental techniques are identical to those previously described.$^{19,20}$

The experimental protocol was started with urine collections for 2 consecutive 10-minute control periods, with an arterial blood sample (2 mL) taken at the midpoint of each collection period. This was followed by first a low dose (0.1 mg/kg per min) and then a high dose (0.5 mg/kg per min) of intraarterial infusions of DETC. During each dose infusion, an initial 5-minute stabilization period was allowed before two 10-minute collection periods of urine were made. After the termination of DETC infusion, 10 minutes was allowed for stabilization before the collection of 2 more 10-minute urine samples to assess the recovery of the renal parameters. An intraarterial infusion of NLA was then started at a dose of 50 $\mu$g/kg per min and continued for the duration of the experiment. After 10 minutes of stabilization, two more 10-minute urine samples were collected during NLA infusion alone. Then the infusions of low and high doses of DETC were repeated in the presence of NLA.

At the end of each experiment, the electromagnetic flow probe was calibrated in situ by timed collections of blood into a graduated cylinder from a catheter placed in the renal artery. The kidney was then removed, stripped of all surrounding tissue, blotted dry, and weighed so that the calculated parameters could be expressed per gram of kidney weight. Flame photometry (Instrumentation Laboratory) was used to determine the sodium concentrations in plasma and urine. Inulin concentrations in the samples were determined by the anthrone colorimetric technique (Gilford Instruments).

Values are reported as mean±SE. Statistical comparisons of differences in the responses were conducted with the use of ANOVA, followed by Newman-Keuls test. Differences in the mean values were deemed significant at $P<0.05$.

**Results**

**Responses to Infusion of DETC on Renal Hemodynamics and Excretory Function Before NLA Administration**

The table summarizes the results in absolute mean values obtained in 6 dogs. The values are the average of the values obtained in two 10-minute collection periods. The percent changes in the responses are illustrated in Figures 1 through 4. Intrarenal infusions of both low and high doses of DETC did not cause any significant change in systemic arterial pressure (Table). Renal vascular resistance (RVR) did not change during low-dose infusion of DETC but increased significantly during high-dose infusion (Table and Figure 1). Low dose of DETC failed to cause any significant change in total RBF (−2±2%), cortical blood flow (CBF, −3±3%), and medullary blood flow (MBF, 0.3±5%); however, high dose caused reductions of 21±5% in RBF, 20±5% in CBF, and 21±7% in MBF (Figures 1 and 2, Table). GFR did not alter significantly during infusion of these DETC doses.
Both low- and high-dose infusions of DETC caused RVR and decreases in RBF, CBF, and MBF (Figures 1 and 2, before NLA infusion did not cause any effects on renal responses in percent changes. Although low-dose DETC absolute mean values, and Figures 1 through 4 illustrate the responses to DETC. The Table summarizes the responses in presence of NLA, there was enhancement of the renal summarized results have been given in the Table. In the cessation of the DETC infusion. The values in the renal response to DETC were seen reversed within 30 minutes of changes in renal hemodynamics and excretory function in different from values obtained during control periods.（Table）.

Responses to DETC on Renal Hemodynamics and Excretory Function During NLA Administration

During inhibition of NO synthase by intraarterial infusion of NLA, there were increases in arterial pressure and RVR and decreases in RBF, CBF, MFB, urine flow, UNaV, and FE Na, without changes in the GFR, as reported previously.17,18 The summarized results have been given in the Table. In the presence of NLA, there was enhancement of the renal responses to DETC. The Table summarizes the responses in absolute mean values, and Figures 1 through 4 illustrate the responses in percent changes. Although low-dose DETC before NLA infusion did not cause any effects on renal hemodynamics, it caused increases in arterial pressure and RVR and decreases in RBF, CBF, and MFB (Figures 1 and 2, Table). Both low- and high-dose infusions of DETC caused an increase in RVR (27±8% and 73±15%) and decreases in RBF (19±4% and 39±4%), CBF (20±6% and 32±5%), and MFB (19±7% and 34±6%), respectively, during NLA infusion. There were dose-dependent marked reductions in GFR (31±9% and 59±7%) during infusion of the doses of DETC in NO synthase–blocked dogs (Figure 3). Greater reductions in urine flow (57±6% and 78±5%) and UNaV (53±9% and 67±10%) were also observed in response to DETC doses infusion in the presence of NLA (Figure 4).

Discussion

The present investigation demonstrates that intraarterial administration of a SOD inhibitor, DETC, in anesthetized dogs would enhance O2− activity in the kidney and result in an increase in RVR and reductions in the basal levels of RBF, urine flow, and UNaV. These changes to DETC were greatly enhanced during NLA infusion, indicating that NO interacts with O2− to prevent its renal vasoconstriction as well as antidiuretic and antinatriuretic effects. DETC, a copper cheating agent, has been used in many previous studies to examine the role of enhanced O2− activity in the biological tissues.15,16,21–23 It was shown that the high dose of DETC used in the present study was effective in producing 80% inhibition of SOD activity.24 In an in vitro study, Pagano et al25 demonstrated the O2− levels (measured by leuigenin chemiluminescence assay) in isolated blood vessels increased nearly 10-fold during application of 10 mmol/L DETC. In a separate in vitro study using isolated rat aorta, we also observed that application of DETC (10 mmol/L) caused an increased level of O2−, which was reversed by the addition of tempol (3 mmol/L) (Nishiyama A, Shokoji T, Abe Y, unpublished observation, 2001).

In the present study, it is noted that low-dose infusion of DETC before NLA infusion did not cause appreciable changes in RBF or GFR but was able to cause substantial reductions in urine flow and UNaV. These results indicated that O2− activity in the kidney may directly influence the tubular reabsorptive function without concomitant changes in renal hemodynamics. During NO inhibition, there was a marked reduction in GFR in response to DETC administration, which was not observed before NLA infusion (Figure 3). These findings strongly suggest that NO provides a renoprotective function against the effects of O2− to maintain the basal filtration rate in the glomerulus.
Our findings in anesthetized dogs are in agreement with those of a recent study in rats by Zou et al.16 who demonstrated that intramedullary administration of DETC at a rate of 0.5 mg/kg per min also caused vasoconstrictor and antinatriuretic effects. They also examined the biomedicopathways responsible for O2− production in the kidney and observed that the outer medullary and cortical regions contain all the major O2−-producing enzymes, such as NADH/ NADPH oxidase and mitochondrial respiratory chain enzyme. Our findings that DETC administration resulted in both cortical and medullary vasoconstriction suggest that enhancement of O2− activity induced by DETC occurred in both the cortical and medullary regions in the kidney. In the present study, simultaneous evaluation of the responses to enhancement of O2− activity on total and regional blood flows, GFR, and renal excretory function has been made more comprehensively in the presence and absence of NO synthase blockade in the kidney.

It is generally believed that O2−-induced vasoconstriction is mainly caused by abolition of NO-mediated vasodilation, as both these oxygen radicals interact with each other.14,15 However, we have observed that renal vasoconstrictor responses to DETC infusion were greatly enhanced in the absence of NO generation. This finding clearly indicates that O2− can exert renal vasoconstrictor effects independent of NO mechanism. The exact mechanism by which O2− can induce vasoconstriction is not yet clear. However, it was reported that an excessive intracellular calcium accumulation could be observed in myocardium because of oxidant stress during ischemic condition in heart.26–28 Thus, it is possible that an increase in intracellular calcium level induced by O2− generation in vascular smooth muscles can cause such direct vasoconstrictor effect. Our results also indicate that O2− may have an influence on renal tubular reabsorption independent of NO, as the effects of DETC on urine flow and UaV were seen to be markedly enhanced during NO synthase inhibition. Thus, it is clear that NO and O2− exert a reciprocal effect on renal tubular reabsorptive function, as NO generally induces diuretic and natriuretic responses in the kidney.16,20 The mechanism involved in the tubular effect of O2− is not yet clear. Further studies are needed to characterize the exact nature of O2− involvement on the vascular and tubular function in the kidney.

The cellular levels of NO and O2− production and their interactions are believed to have a major impact on the expression of signaling mechanisms that control vascular reactivity.15,22 It is conceivable that the biological stability of NO and its signaling mechanisms are more likely to be controlled by the levels of O2− generated within the cellular tissue. Omar et al.13 have demonstrated that the relaxation of endothelium-removed bovine coronary arteries to a NO donor is attenuated by pretreatment with an inhibitor of SOD, and this effect is prevented by a scavenger of intracellular O2−. The findings in that study21 show how the scavenging of endogenous O2− by SOD protects NO from being inactivated by this oxidant. Thus, it is conceivable that if there is an imbalance in the production of NO and O2−, the major signaling effect observed is likely to be an alternations in NO-elicited vascular relaxing and other effects.14 Apart from its vascular relaxing effects, NO also regulates other cellular mechanisms such as prostaglandin production by vascular endothelium,21,28 mitochondrial oxygen metabolism,14 cellular membrane ionic channels, and their dependent signaling mechanisms15 and, particularly in the kidney, regulates the ionic channel activity in epithelial cells involved in tubular reabsorptive mechanism.30 Thus, processes originating from the interaction of O2− with NO have the potential to influence the endothelial, epithelial, and other cellular functions in the organs.

In conclusion, the results of the present investigation indicate that O2− exerts renal vasoconstriction and tubular effects, leading to salt and water retention. These data are consistent with the hypothesis that NO serves an important protective role against the actions of O2− in the kidney.

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