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Role of Nitric Oxide in Lipopolysaccharide-Induced Oxidant Stress in the Rat Kidney

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ABSTRACT. Lipopolysaccharide (LPS)-induced renal oxidant injury and the role of nitric oxide (NO) were evaluated using the inducible nitric oxide synthase (iNOS) inhibitor L-iminoethyl-lysine (L-NIL). One group of male rats received LPS (*Salmonella minnesota*; 2 mg/kg, i.v.). A second group received LPS plus L-NIL (3 mg/kg, i.p.). A third group received saline i.v. At 6 hr, iNOS protein was induced in the kidney cortex, and plasma nitrate/nitrite levels were increased from 4 ± 2 nmol/mL in the Saline group to 431 ± 23 nmol/mL in the LPS group. The value for the LPS + L-NIL group was reduced significantly to 42 ± 9 nmol/mL. LPS increased blood urea nitrogen levels from 13 ± 1 to 47 ± 3 mg/dL. LPS + L-NIL reduced these levels significantly to 29 ± 2 mg/dL. Plasma creatinine levels were unchanged in all groups. Tissue lipid peroxidation products in the kidney were increased from 0.16 ± 0.01 nmol/mg in the Saline group to 0.30 ± 0.03 nmol/mg in the LPS group. LPS + L-NIL reduced the values significantly to 0.22 ± 0.02 nmol/mg. Intracellular glutathione levels were decreased in the kidneys from 1.32 ± 0.1 nmol/mg in the Saline group to 0.66 ± 0.08 nmol/mg in the LPS group. LPS + L-NIL increased the levels significantly to 0.99 ± 0.13 nmol/mg. LPS increased the 3-nitrotyrosine-protein adducts in renal tubules as detected by immunohistochemistry, indicating the generation of peroxynitrite. L-NIL decreased adduct formation. These data indicated that LPS-induced NO generation resulted in peroxynitrite formation and oxidant stress in the kidney and that inhibitors of iNOS may offer protection against LPS-induced renal toxicity. BIOCHEM PHARMACOL **59**;2:203–209, 2000. © 1999 Elsevier Science Inc.

KEY WORDS. nitric oxide; peroxynitrite; L-N^6-(1-iminoethyl)-lysine; lipopolysaccharide; glutathione; kidney; oxidant stress

LPS†, a component of the Gram-negative bacterial cell wall released during septicemia, is a major cause of septic shock in humans [1]. LPS triggers the synthesis and release of cytokines and the vasodilator NO. A major contributor to the increase in NO production is LPS-stimulated expression of iNOS. This occurs in the vasculature and most organs including the kidney [2–4]. Septicemia and septic shock are associated with high mortality, and current therapy is mostly supportive and largely ineffective [1]. Acute renal failure is a serious complication of septicemia and septic shock [5, 6]. Although hypotension and reduced renal blood flow can contribute to renal failure, animal models have shown that LPS can cause renal injury in the absence of significant falls in systemic blood pressure or renal blood flow [7].

In addition to increasing the synthesis of NO, LPS also causes the synthesis of reactive oxygen species such as superoxide in the lung [8, 9], liver [10], and kidney [11, 12]. In the rat, LPS causes a progressive decline in renal function that can be delayed by administration of SOD or dimethylthiourea [11] or attenuated by inhibition of NO synthesis [7]. However, the roles of NO and oxidants in the development of renal failure are unknown.

NO and superoxide react spontaneously to form the potent and versatile oxidant $ONOO^-$ that can react with lipids, proteins, and DNA [13, 14]. $ONOO^-$ is difficult to measure *in vivo* due to its short half-life [15]. However, the formation of 3-nitrotyrosine-protein adducts is a reliable biomarker of $ONOO^-$ generation [16], and specific immunochemical assays have been developed [17, 18]. NO-mediated oxidant stress and $ONOO^-$ formation have never been examined in LPS-induced kidney injury. However, plasma nitrotyrosine levels are elevated in patients with septic shock [19].

Studies were performed in an endotoxemia model that would allow determination of whether oxidant injury occurs in the kidney prior to the development of renal failure and would make it possible to examine the role of NO in the development of oxidant stress. The role of NO was evaluated using the selective iNOS inhibitor L-NIL, and

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† *Abbreviations*: LPS, lipopolysaccharide; NO, nitric oxide; iNOS, inducible nitric oxide synthase; $ONOO^-$, peroxynitrite; L-NIL, L-iminoethyl-lysine; SOD, superoxide dismutase; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); TBARS, thiobarbituric acid reactive substances; and BUN, blood urea nitrogen.

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$ONOO^-$ formation was examined using immunohistochemistry.

MATERIALS AND METHODS

Materials

LPS (*Salmonella minnesota*), phenylmethylsulfonyl fluoride, DTNB, GSH, 2-thiobarbituric acid, 1,1,3,3-tetraethoxypropane, and leupeptin were purchased from the Sigma Chemical Co. L-N^6-(1-Iminoethyl)-lysine hydrochloride was purchased from the Alexis Corp. Anti-iNOS rabbit antibody was purchased from the Cayman Chemical Co. Anti-rabbit peroxidase-conjugated antibody and an enhanced chemiluminescence (ECL) detection kit were purchased from Amersham International. A Vectastain Elite peroxidase ABC kit was purchased from Vector Laboratories, Inc. Rabbit anti-nitrotyrosine antibody was purchased from Upstate Biotechnology. A NO assay kit was purchased from Oxford Biochemical Research, Inc. All other chemicals were of reagent grade and were purchased from Sigma.

LPS-Induced Injury

Male Sprague-Dawley rats (250 g) were divided into three groups. One group received 2 mg/kg of LPS, i.v., under ethyl ether anesthesia (LPS group). A second group received the same dose of LPS plus two doses of L-NIL at 3 mg/kg, i.p. (LPS + L-NIL group). One dose of L-NIL was given at the time of LPS administration and the second dose 3 hr later. A third group received saline and served as a control (Saline group). All injections were made at a volume of 1 mL/kg. At 6 hr, animals were anesthetized with pentobarbital sodium (50 mg/kg). Blood was collected in heparinized syringes. Then kidneys were perfused to remove blood with 10 mL of modified Krebs buffer containing: 103 mM NaCl, 5.0 mM KCl, 2.0 mM NaH_2PO_4, 1.0 mM $MgSO_4$, 1.0 mM $CaCl_2$, 5.0 mM glucose, 5.0 mM malate, 5.0 mM glutamate, 4.0 mM lactate, 1.0 mM alanine, 20 mM $NaHCO_3$, and 10 mM HEPES (pH 7.4). For lipid peroxidation product determination and western blot analysis of iNOS, the kidneys were excised, and the cortex was dissected and frozen rapidly in liquid nitrogen. For intracellular GSH determination, whole kidneys were removed and frozen in liquid nitrogen. In some animals, one kidney was removed and fixed in 10% phosphate-buffered formalin for immunohistochemistry.

Measurement of BUN and Creatinine

Plasma BUN and creatinine concentrations were analyzed using a Beckman Synchron CX7 Analyzer.

iNOS Western Blot Analysis

A rabbit polyclonal anti-iNOS antibody (Cayman Chemical) was used to detect iNOS protein. Tissue homogenates (100 μg protein/lane) were separated by SDS-PAGE (7.5%), and then transferred to nitrocellulose using an electroblotting transfer apparatus. Nitrocellulose membranes were incubated in blocking buffer (10 mM Tris, 100 mM NaCl, 0.1% Tween 20, and 5% non-fat milk) overnight at 4°. Then they were incubated for 90 min at room temperature with primary antibody rabbit polyclonal anti-iNOS diluted (1:500) in blocking buffer. The membranes were washed three times for 10 min in washing buffer (10 mM Tris, 100 mM NaCl, and 0.1% Tween 20), then incubated for 60 min at room temperature with secondary antibody (anti-rabbit peroxidase-conjugated antibody) diluted in blocking buffer. Finally, the membranes were washed and developed using the ECL detection kit as described by the manufacturer.

Measurement of Plasma Nitrite and Nitrate (NO_2^-/NO_3^-) Levels

Plasma samples (50 μL) were deproteinized by incubating with 140 μL of deionized H_2O and 10 μL of 30% $ZnSO_4$ at room temperature for 15 min, and then were centrifuged at 2000 *g* for 10 min. Nitrate was converted to nitrite using cadmium beads, and nitrite was measured spectrophotometrically using an NO assay kit as described by the manufacturer.

Determination of Intracellular GSH Equivalents

Tissues were homogenized and deproteinized in buffer containing NaH_2PO_4 (125 mM), EDTA (6.3 mM), and sulfosalicylic acid (5%) in a 1:5 ratio (wet weight to volume). Total GSH equivalents were determined using published methods [20]. After centrifugation (15,000 *g* for 5 min), the supernatant was neutralized with triethanolamine to a pH of 7.0 to 7.4. Stock solutions of NADPH (0.3 mM), DTNB (6 mM), and glutathione reductase (50 U/mL) were made in buffer containing NaH_2PO_4 (125 mM) and EDTA (6.3 mM) at pH 7.4. The assay mixture contained 700 μL of NADPH solution, 100 μL of DTNB solution, and 10 μL of glutathione reductase solution. The reaction was initiated by the addition of 200 μL of test sample. The rate of reduction of DTNB was determined by monitoring continuously the absorbance at 412 nm for 3 min. A standard curve was prepared using pure GSH standards. Data were expressed as GSH equivalents per milligram of wet tissue.

Determination of Lipid Peroxidation Products

Lipid peroxidation products were determined by measuring the levels of TBARS [20]. Kidney cortex was homogenized in buffer containing 0.25 M sucrose, 1 mM EDTA, and 10 mM HEPES, and then was centrifuged for 5 min at 2000 *g*. The protein concentration of the resulting supernatant was adjusted to 2 mg/mL, then deproteinized using 10% trichloroacetic acid and centrifuged for 5 min at 12,000 *g*. The supernatant (900 μL) was combined with 500 μL of 2-thiobarbituric acid reagent (0.78%), heated in a boiling

water bath for 10 min, and cooled on ice. Samples were warmed to room temperature, and the absorbance was measured at 532 nm. A standard curve was prepared from 1,1,3,3-tetraethoxypropane. Standards and samples gave the expected peak absorbance at 532 nm for TBARS adducts. Data were expressed as total TBARS per milligram of protein.

Detection of 3-Nitrotyrosine-Protein Adducts

Immunohistochemistry for 3-nitrotyrosine-protein adducts was performed using a rabbit polyclonal anti-nitrotyrosine antibody from Upstate Biotechnology. Paraffin-embedded tissue sections (3 μm) were cleared in xylene, rehydrated, and washed in PBS. Endogenous peroxidase activity was blocked by incubation with 1% H_2O_2 in methanol, and nonspecific protein binding was blocked by incubation with 10% goat serum in PBS. Rabbit anti-nitrotyrosine antibody (1:100 dilution) was incubated with the sections for 1 hr at room temperature. Primary antibody was detected using the Vectastain Elite peroxidase ABC kit with 3,3′-diaminobenzidine as substrate. A brown precipitate forms where the anti-nitrotyrosine antibody binds the tissue section. Gill's hematoxylin was used as a counterstain. As a negative control, the antigenic binding site of the anti-nitrotyrosine antibody was blocked by preincubation of the antibody with 3-nitrotyrosine (10 mM) for 1 hr at room temperature.

Renal Histology

Tissue sections were stained with hematoxylin and eosin for evaluation of disseminated intravascular coagulation and tubular morphology in a blinded fashion by a renal pathologist.

Data Analysis

Data are reported as means ± SEM. Each N represents data or tissue obtained from one rat. All data were analyzed by one-way ANOVA followed by the Student–Newman–Keuls test. $P < 0.05$ was considered statistically significant.

RESULTS

Plasma Creatinine and BUN Levels

We chose an endotoxemia model that would allow us to examine NO-mediated renal toxicity prior to the development of renal failure. In preliminary studies, we established that 6 hr following a 2 mg/kg dose of LPS, induction of iNOS in the kidney occurred without apparent renal failure. Plasma creatinine levels are a frequently used marker of renal failure. At 6 hr following LPS administration, plasma levels of creatinine in all groups were not significantly different ($P > 0.05$). The levels of plasma creatinine for the Saline, L-NIL, LPS, and LPS + L-NIL groups were 0.29 ± 0.03, 0.33 ± 0.03, 0.52 ± 0.1, and 0.42 ± 0.06 mg/dL, respectively.

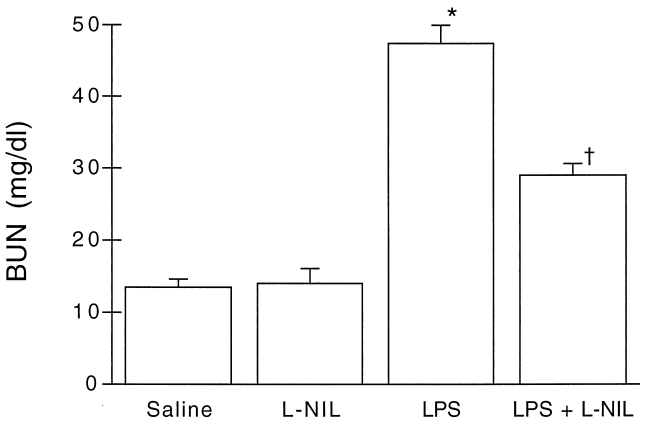

FIG. 1. Effect of L-NIL on BUN levels after LPS administration. BUN levels were measured 6 hr after treatment with saline, L-NIL (3 mg/kg, i.p., two doses, 3 hr apart), LPS (2 mg/kg, i.v.), or LPS (2 mg/kg, i.v.) plus L-NIL (3 mg/kg, i.p., two doses, 3 hr apart). Data are means ± SEM (N = 3 for the L-NIL group and N = 8 for all other groups). Key: (*) $P < 0.05$ compared with the Saline group and (†) $P < 0.05$ compared with all other groups.

One hallmark of endotoxemia is a rise in BUN due to increased protein catabolism [21]. Six hours following LPS administration, BUN concentration increased from 13 ± 1 mg/dL in the Saline group to 47 ± 3 mg/dL in the LPS group ($P < 0.05$) (Fig. 1). The value for the LPS + L-NIL group was 29 ± 2 mg/dL ($P < 0.05$ compared with the Saline and LPS groups). The value for the L-NIL alone group was 14 ± 2 mg/dL and was not different from the Saline group. These data show that L-NIL provided partial protection against LPS-induced protein catabolism. Hematocrit was unchanged in all groups (data not shown).

Western Blot Analysis of iNOS

The presence of iNOS protein in the kidney cortex was detected using western blot analysis with an anti-iNOS antibody. These data are presented in Fig. 2. iNOS protein could not be detected in kidneys from animals in the Saline group. However, kidneys from animals in both the LPS and LPS + L-NIL groups expressed iNOS protein. Treatment with L-NIL did not appear to affect iNOS protein expression.

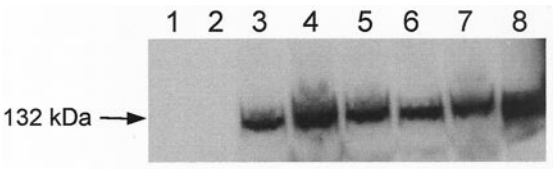

FIG. 2. Western blot analysis of iNOS in kidney 6 hr after LPS administration. Lanes 1 and 2, Saline group; lanes 3–5, LPS group (2 mg/kg, i.v.); and lanes 6–8, LPS (2 mg/kg, i.v.) + l-NIL group (3 mg/kg, i.p., two doses, 3 hr apart). Each lane contains protein (100 μg) from a separate animal.

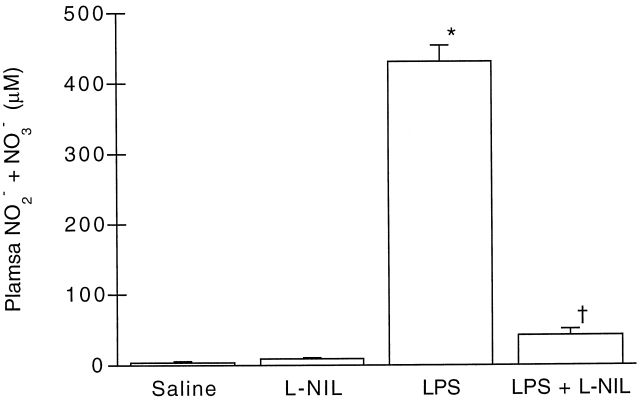

FIG. 3. Effect of l-NIL on plasma NO_2^-/NO_3^- concentration after LPS administration. Plasma NO_2^-/NO_3^- concentrations were measured 6 hr after treatment with saline, L-NIL (3 mg/kg, i.p., two doses, 3 hr apart), LPS (2 mg/kg, i.v.), or LPS (2 mg/kg, i.v.) plus L-NIL (3 mg/kg, i.p., two doses, 3 hr apart). Data are means ± SEM (N = 3 for the L-NIL group and N = 4 for all other groups). Key: (*) $P < 0.05$ compared with the Saline group and (†) $P < 0.05$ compared with all other groups.

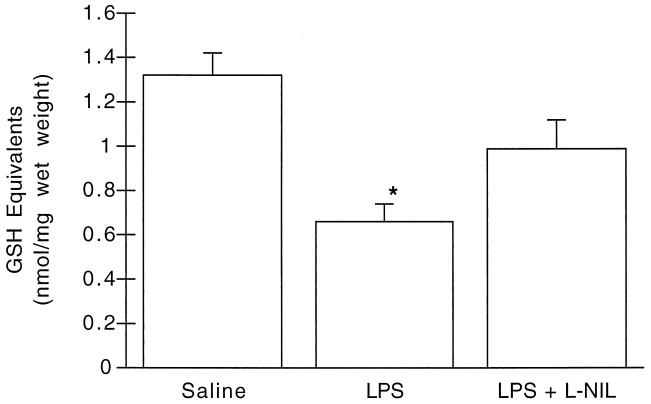

FIG. 4. Effect of LPS on intracellular GSH concentration in the whole kidney. Intracellular GSH equivalents were measured in the whole kidney 6 hr after treatment with saline, LPS (2 mg/kg, i.v.), or LPS (2 mg/kg, i.v.) plus L-NIL (3 mg/kg, i.p., two doses, 3 hr apart). Data are means ± SEM (N = 4–7). Key: (*) $P < 0.05$ compared with all other groups.

Measurement of Plasma NO_2^-/NO_3^- Concentration

NO_2^- and NO_3^- are the metabolic products of NO and are convenient markers of NO formation. NO_2^-/NO_3^- concentration was measured in plasma 6 hr after treatments (Fig. 3). LPS increased the levels of plasma NO_2^-/NO_3^- from 4 ± 2 nmol/mL in the Saline group to 431 ± 23 nmol/mL in the LPS group ($P < 0.05$). In the LPS + L-NIL group, NO_2^-/NO_3^- concentration was decreased significantly to 42 ± 9 nmol/mL ($P < 0.05$ compared with the Saline and LPS groups). The value for the L-NIL alone group was 9 ± 2 nmol/mL and was not different from the Saline group. Although L-NIL did not affect iNOS expression after LPS, it did inhibit NO synthesis.

Intracellular GSH Equivalents

Several studies suggest that LPS can induce oxidant stress in the kidney [11, 20]. We measured two markers of oxidant stress in the kidney 6 hr following LPS administration. The first was intracellular GSH concentration (Fig. 4). GSH is an important cellular defense against oxidant injury [22]. LPS decreased GSH equivalents in the whole kidney from 1.32 ± 0.1 nmol/mg in the Saline group to 0.66 ± 0.08 nmol/mg ($P < 0.05$) in the LPS group. In the LPS + L-NIL group the level was 0.99 ± 0.13 nmol/mg ($P < 0.05$ compared with the LPS group), a value not different from the Saline group.

Tissue Lipid Peroxidation Products

Lipid peroxidation in the kidney cortex was used as a second marker of oxidant injury (Fig. 5). The TBARS assay [23] measures membrane lipid peroxidation breakdown products (aldehydes, such as malondialdehyde, and ketones). The basal level of TBARS in the kidney cortex was 0.16 ± 0.01 nmol/mg in the Saline group. In the LPS group, the value was increased to 0.30 ± 0.03 nmol/mg ($P < 0.05$). In the LPS + L-NIL group, the value was 0.22 ± 0.02 nmol/mg ($P < 0.05$ compared with the LPS group) and was not different from the Saline group. These data suggest that increased NO synthesis triggered by LPS results in oxidant stress in the kidney.

Immunohistochemical Detection of 3-Nitrotyrosine-Protein Adduct Formation

An apparent NO-mediated oxidant injury in the kidney following LPS administration suggests the generation of $ONOO^-$. Immunohistochemical detection of 3-nitrotyrosine-protein adduct formation is a reliable biomarker of $ONOO^-$ generation [16]. Representative photographs of the immunohistochemical analyses performed on paraffin-embedded sections from whole kidney obtained 6 hr fol-

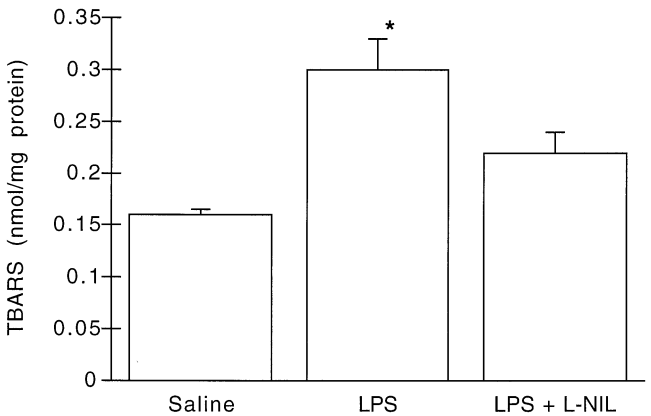

FIG. 5. Effect of LPS on lipid peroxidation in the kidney cortex. Lipid peroxidation products (TBARS) were measured in the perfused kidney cortex 6 hr after treatment with saline, LPS (2 mg/kg, i.v.), or LPS (2 mg/kg, i.v.) plus L-NIL (3 mg/kg, i.p., two doses, 3 hr apart). Data are means ± SEM (N = 4–7). Key: (*) $P < 0.05$ compared with all other groups.

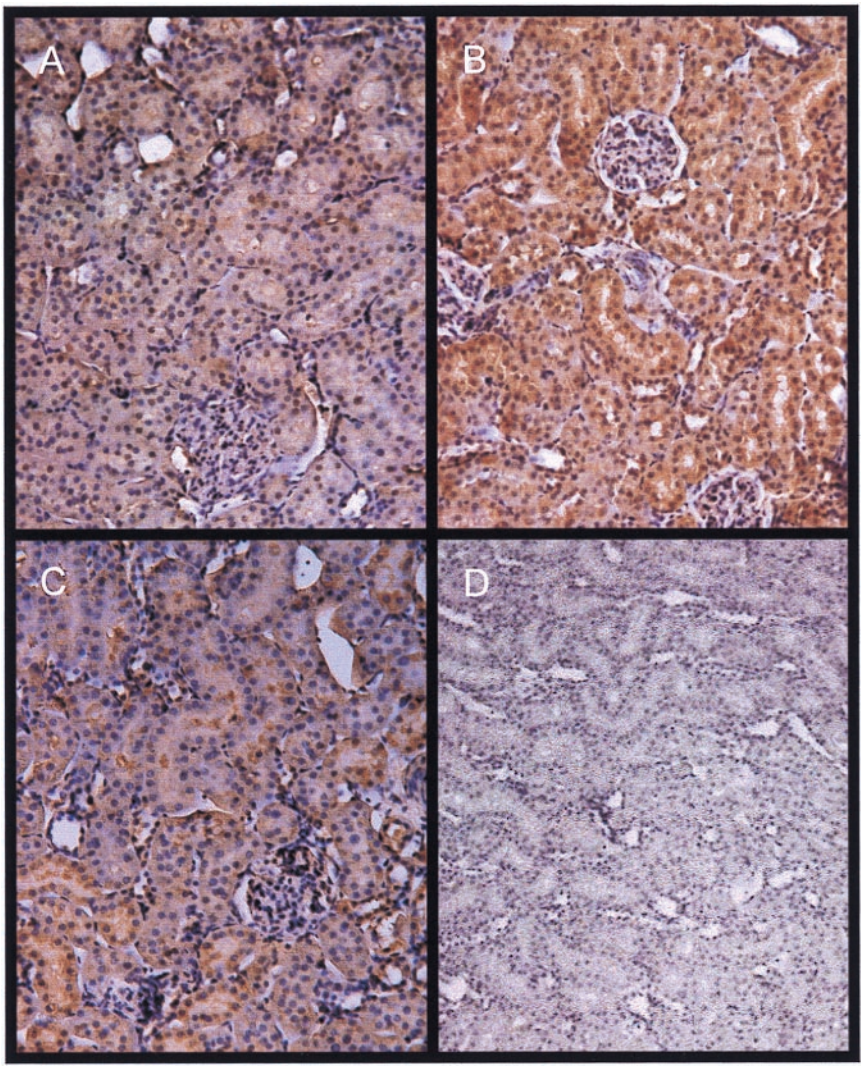

FIG. 6. Immunohistochemical detection of 3-nitrotyrosine-protein adducts in the kidney after LPS administration. Shown are representative photographs of immunohistochemical detection of 3-nitrotyrosine-protein adducts performed on kidney sections isolated 6 hr after treatment with saline, LPS (2 mg/kg, i.v.), or LPS (2 mg/kg, i.v.) + L-NIL (3 mg/kg, i.p., two doses, 3 hr apart). Panel A: Saline group (original magnification = 200x). Panel B: LPS group (original magnification = 200x). Panel C: LPS + L-NIL group (original magnification = 200x). The brown stain represents detection of 3-nitrotyrosine-protein adducts. Panel D: section from the LPS group (original magnification = 100x) detected with antibody preincubated with 10 mM 3-nitrotyrosine to block antigenic activity. Photographs are representative of results from three animals.

lowing treatment are presented in Fig. 6. A brown precipitate indicates the presence of 3-nitrotyrosine-protein adducts. Sections from the Saline group displayed very low levels of diffuse staining (panel A). Sections from the LPS group showed intense staining in tubules but not in the glomeruli (panel B). Sections from the LPS +L-NIL group showed an intermediate level of staining, indicating that L-NIL treatment decreased the relative levels of adducts (panel C). The antigenic binding specificity of the anti-nitrotyrosine antibody was confirmed by blocking the antigen binding site with 3-nitrotyrosine prior to its addition to the tissue section (panel D). No staining was detected in sections from the LPS group when treated with the blocked antibody. The results suggest that $ONOO^-$ is formed in the kidney following LPS administration.

Renal Histology

Renal histology was evaluated on hematoxylin and eosin-stained tissue sections. No evidence of disseminated intravascular coagulation was observed in any of the treated animals. Also, there was essentially no evidence of tubular necrosis.

DISCUSSION

Septicemia and septic shock are associated with high mortality, and current therapy is mostly supportive and largely ineffective [1]. Pulmonary, hepatic, and renal failures are frequent and serious complications [5, 6]. Although hypotension and reduced renal blood flow can contribute to renal failure, animal models show that LPS can cause renal injury even in the absence of significant falls in systemic blood pressure or renal blood flow [7]. The potential for direct toxicity of LPS to renal cells *in vivo* is supported by *in vitro* studies showing that the biologically active component of LPS, lipid A, produces NO-mediated oxidant injury to renal proximal tubules [20]. Whereas NO is clearly important in the pathogenesis of septicemia, the mechanisms of NO-mediated injury in the kidney are unclear.

Nonselective NOS inhibitors (e.g. *N*-monomethyl-L-arginine and *N*-nitro-L-arginine methyl ester) inhibit both

constitutive endothelial NOS and iNOS. Although these inhibitors can raise blood pressure, they are detrimental in the treatment of septic shock, because they can cause coronary vasoconstriction, decreased cardiac output, pulmonary hypertension, and renal thrombosis [24–27]. These effects are presumably due to inhibition of endothelial NOS. In the case of renal thrombosis, inhibition of endothelial cell and platelet NOS by nonselective NOS inhibitors could remove a protective effect of NO [27]. For these reasons, selective inhibition of iNOS could be beneficial. l-NIL is reported to be a selective inhibitor of iNOS [28] and has been used to evaluate the role of iNOS in a number of *in vivo* studies [29, 30]. In our model of endotoxemia, the dosing regimen used for L-NIL inhibited LPS-stimulated plasma NO_2^-/NO_3^- concentration by 90% and reduced protein catabolism and oxidant stress in the kidney.

Stimulation of superoxide generation in response to LPS has been suggested in lung, liver, and kidney, as has its involvement in organ injury [9–11]. The present studies suggest that, in the kidney, superoxide may act, at least in part, through the generation of $ONOO^-$. NO and superoxide undergo a bi-radical addition reaction to yield $ONOO^-$. The rate constant for the reaction of NO with superoxide ($6.7 \times 10^9\ M^{-1}\ sec^{-1}$) is faster than that for superoxide reacting with SOD ($2.0 \times 10^9\ M^{-1}\ sec^{-1}$) [31]. Thus the formation of $ONOO^-$ is favored in conditions where both NO and superoxide are formed. $ONOO^-$ is a potent oxidant thought to participate in oxidant injury associated with chronic inflammation [18, 32], acute lung injury [33], and LPS-induced liver injury [34]. Thus, the protective effects of SOD and reactive oxygen scavengers on LPS-induced renal failure [11] may be due, in part, to inhibition of $ONOO^-$-mediated toxicity [31, 35]. l-NIL provided significant but not complete protection. This could be due to the incomplete inhibition of iNOS, NO synthesis by other isoforms, and/or the unmasking of the toxicity of superoxide alone.

$ONOO^-$ formation has been detected in several models of injury that are mediated by reactive oxygen species. 3-Nitrotyrosine-protein adducts have been identified in the myocardium following ischemia-reperfusion injury [36] and in patients with myocarditis [32]. Plasma levels of nitrotyrosine also are elevated in patients with septic shock [19] and in livers from mice treated with LPS [34]. The presence of 3-nitrotyrosine-protein adducts in the kidney provides the first evidence of coincident generation of NO and superoxide in the kidney following LPS administration.

GSH and glutathione peroxidase constitute a major defense system against $ONOO^-$-mediated oxidations [22, 37]. In the present study, LPS caused a fall in kidney GSH levels and a rise in lipid peroxidation products, two markers of oxidant injury. L-NIL reduced oxidant injury and apparent peroxynitrite formation significantly. These data suggest that NO and $ONOO^-$ contribute to the development of oxidant injury. Furthermore, the source of NO may be iNOS. However, other isoforms of NOS are expressed by the kidney, and their activity may increase following LPS administration [38]. The cell types responsible for NO and superoxide generation in the kidney in response to LPS are not known. Interestingly, proximal tubule constitutive NOS and iNOS are both capable of generating superoxide in addition to NO [39, 40].

In summary, at 6 hr following LPS administration, the rat kidney showed signs of oxidant stress that were ameliorated by the iNOS inhibitor l-NIL. The presence of 3-nitrotyrosine-protein adducts in the kidney indicated the generation of both NO and superoxide and the formation of the strong oxidant $ONOO^-$. Coincident generation of NO and superoxide results in the generation of reactive nitrogen and oxygen species. LPS could cause a heightened environment for oxidant stress that overcomes cellular defense mechanisms, leaving the kidney susceptible to oxidant injury. Reactive nitrogen species should be considered potential targets for therapeutic intervention. iNOS inhibitors may offer an advantage over antioxidants in that they could improve hemodynamics as well as reduce the formation of $ONOO^-$.

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