Molecular and Cellular Biochemistry **271:** 107–112, 2005.

The effects of nitric oxide synthesis on the Na^+,K^+-ATPase activity in guinea pig kidney exposed to lipopolysaccharides

Ilgim Seven,[1] Nurten Türközkan[1] and Behzat Çimen[2]

[1]*Department of Biochemistry, Faculty of Medicine, Gazi University, Ankara;* [2]*Department of Chemistry–Biochemistry, Faculty of Science–Art, Kahramanmaras Sütçü Imam University, K. Maras, Turkey*

Received 7 September 2004; accepted 29 October 2004

Abstract

Endotoxins (lipopolysaccharides; LPS) are known to cause multiple organ failure, including renal dysfunction. LPS triggers the synthesis and release of cytokines and the vasodilatör nitric oxide ($NO^\bullet$). A major contributor to the increase in $NO^\bullet$ production is LPS-stimulated expression of inducible nitric oxide synthase (iNOS). This occurs in vasculature and most organs including the kidney. During endotoxemia, $NO^\bullet$ and superoxide react spontaneously to form the potent and versatile oxidant peroxynitrite ($ONOO^-$) and the formation of 3-nitrotyrosine (nTyr)-protein adducts is a reliable biomarker of $ONOO^-$ generation. Therefore, the present study was aimed at investigating the role of endogenous nitric oxide in regulating Na^+,K^+-ATPase activity in the kidney, and at investigating the possible contribution of reactive nitrogen species (RNS) by measuring of iNOS activity. In addition, the present study was aimed at investigating the relationship between nTyr formation with iNOS and Na^+,K^+-ATPase activities. Previously in our study, nTyr was not detectable in kidney of normal control animals but was detected markedly in LPS exposed animals. In this study, kidney Na^+,K^+-ATPase activity were maximally inhibited 6 h after LPS injection (P:0.000) and LPS treatment significantly increased iNOS activity of kidney (P:0.000). The regression analysis revealed a very close correlation between Na^+,K^+-ATPase activity and nTyr levels of LPS treated animals ($r = -0.868$, $P = 0.001$). Na^+,K^+-ATPase activity were also negatively correlated with iNOS activity ($r = -0.877$, $P = 0.001$) in inflamed kidney. These data suggest that $NO^\bullet$ and $ONOO^-$ contribute to the development of oxidant injury. Furthermore, the source of $NO^\bullet$ may be iNOS. iNOS are expressed by the kidney, and their activity may increase following LPS administration. In addition, $NO^\bullet$ and $ONOO^-$ formation inhibited Na^+,K^+-ATPase activity. This results also have strongly suggested that bacterial LPS disturbs activity of membrane Na^+,K^+-ATPase that may be an important component leading to the pathological consequences such as renal dysfunction in which the production of RNS are increased as in the case of LPS challenge. (Mol Cell Biochem **271:** 107–112, 2005)

Key words: 3-nitrotyrosine, Na^+,K^+-ATPase, iNOS, kidney, guinea pig, LPS

Introduction

LPS, the cell wall component of many bacteria including *Escherichia coli* (*E. coli*), can induce many of the host defenses required for bacterial killing and up-regulates nitric oxide and cytokine production in single cells, is isolated tissues and in whole animals models. Intraperitoneal injection of LPS induces the expression of iNOS protein in many cells and tissues including kidney. In other words, intraperitoneal LPS treatment up-regulates iNOS and cytokine production in the kidney [1]. Sepsis is a response to infection characterized by the production of inflammatory mediatörs and cytokines, such as TNF-α, IL-1β, IFN-γ, CD14, ICAM-1 [2, 3].

Induction of iNOS in LPS-treated animals is associated with hypotension, reduction in glomerular filtration rate (GFR) [4]. Renal proximal tubule and inner medullary

Address for offprints: Dr. Behzat Çimen, Kahramanmaras Sütçü Imam Üniversitesi, Fen-Edebiyat Fakültesi, Kimya Bölümü-Biyokimya A.D., K. Maras, Turkey (E-mail: bcimen@ksu.edu.tr)

collecting duct cells can produce $NO^{\bullet}$ *via* expression of an inducible isoform of nitric oxide synthase [5].

Acute kidney dysfunction, manifested by a reduction in renal blood flow and in the GFR, is a common finding in septic shock [6]. *E. coli* endotoxin (LPS) can induce renal cortical necrosis [7].

Administration of bacterial LPS produces the clinical syndrome of septic shock by initiating a variety of processes including activation of cytokines. Pathophysiologic consequences include cellular injury and major cardiovascular effects including hypotension, reduction in systemic vascular resistance, and compromise of renal function [4].

An iNOS has recently been described in proximal tubule epithellium [8]. The pathological alterations in the proximal tubules may directly contribute to the reduction in the reabsorption ability of the proximal tubules [9].

$NO^{\bullet}$ and $ONOO^{-}$ contribute to the development of oxidant injury. Isoforms of nitric oxide synthase (NOS) are expressed by the kidney, and their activity may increase following LPS administration. The cell types responsible for $NO^{\bullet}$ 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 $NO^{\bullet}$ [10]. The production of both $NO^{\bullet}$ and superoxide increases in septic shock. The cogeneration of these molecules is known to yield $ONOO^{-}$, which preferentially nitrates tyrosine residues of protein and nonprotein origins [11]. The presence of nTyr in the kidney has been associated with several pathological conditions [12].

Na^{+},K^{+}-ATPase is an energy utilizing transmembrane enzyme, which is responsible for the maintenance of ionic gradients of Na^{+} and K^{+}ATPase has been shown to very susceptible to free radicals and membrane lipid peroxidation [13]. Previously, it has been demonstrated that $NO^{\bullet}$-derived products ($NO_2^{\bullet}$ and $ONOO^{-}$) or SNAP (a $NO^{\bullet}$ donor) or SIN-1 (a $ONOO^{-}$ donor) inhibit Na^{+},K^{+}-ATPase activity *via* the possible oxidation of thiol groups of the enzyme in cerebral cortex [14, 15], erythrocytes [16], kidney proximal tubule [17] and liver plasma membrane [18]. Previously, it has been suggested that peroxynitrite signaling participates in the regulation of renal Na^{+},K^{+}-ATPase activity [17]. In addition, it has been suggested that the endogenous $NO^{\bullet}$ plays a direct inhibitory role on Na^{+},K^{+}-ATPase activity in the kidney [19]. On the other hand, it has been also reported that $NO^{\bullet}$ generated by mouse proximal tubule epithelial cell iNOS inhibits Na^{+},K^{+}-ATPase activity in an autocrine fashion and that this inhibition is accompanied by a reduction in a Na-dependent solute transport [8].

Previously, we also had measured nTyr levels of exposed LPS guinea pig kidney. The aim of this study is to evaluate the effects on Na^{+},K^{+}-ATPase and iNOS activities of LPS-induced free radicals in guinea pig kidney and determine the correlation with nTyr formation of this activation of the enzymes.

Material and methods

Our chemical were obtained from Sigma Chemical (St. Louis, MO) and Merck Chemical (Germany). All the reagents and chemicals used in these experiments were of analytical grade of highest purity. All organic solvents were HPLC grade.

Guinea pigs (300–500 g) were divided into two groups ($n = 10$ each group). Group 1 animals were injected with saline (control group). Group 2 animals were injected with *E. coli* intraperitoneally dosed at 12×10^9 colony-forming U/kg (LPS-treated group). Animals were sacrificed under ether anaesthesia at 6 h after *E. coli* injection [16, 20, 21]. After sacrifice, the kidneys were removed, washed with cold NaCI 0.9% and immediately kept frozen in liquid nitrogen. The kidney tissues were stored at $-70\,^{\circ}$C until use.

Measurement of 3-nitrotyrosine

Kidney sample (0.5 g) in 1.5 ml buffer (50 mM potassium-phosphate buffer, pH 7.4) was homogenised and hydrolysed in 6 N HCI at 90–110 °C for 18–24 h. Hydrolysed samples were centrifugated at 3000 rpm for 10 min, the supernatants were separated for the measurement of the 3-nitrotyrosine levels. The samples were analysed on a Hewlett Packard 1050 diode array detector HPLC apparatus (Hewlett Packard, Waldbron, Germany). The analytical column was 5-μm pore size Spherisorb ODS-2 C18 reverse-phase column (4.6 × 250 mm, Alltech, Dearfield, IL). The guard column was a C18 cartridge (Alltech, Dearfield, IL). The mobile phase was 50 mmol/l sodium acetate/50 mmol/l citrate/8% (v/v) methanol pH 3.1. HPLC analysis was performed under isocratic conditions at a flow rate of 1 ml/min and UV detector set at 274 nm. 3-NT peaks were determined according to its retention time and confirmed by spiking with added exogenous nTyr. Concentrations of nTyr were calculated from nTyr standard curve and expressed as nmol/g tissue [16, 20–22].

Measurement of $Na^{+}K^{+}$ATPase activity

Ten percent kidney homogenate was prepared for the $Na^{+}K^{+}$ATPase study using a glass-homogenizer. Homogenates were centrifuged at 3000 rpm for 5 min and supernatant was separated. $Na^{+}K^{+}$ATPase activity in the supernatant was determined. $Na^{+}K^{+}$ATPase activity was assessed by the measurement of the produced inorganic phosphate and results were expressed as specific activity (μmol P_i/h/mg protein) [16, 21–23].

Assay for nitric oxide synthase

The kidneys were homogenized with 5 vol. of a buffer containing 10 mM HEPES, 0.32 M sucrose, 0.1 mM EDTA, 1 mM dithiothretiol, 10 μg of soyabean tripsin inhibitör per millilitre, 10 μg of leupeptin per millilitre, 2 μg of aprotinin per millilitre and 1 mg of PMSF per millilitre, adjusted to pH 7.4 (at room temperature) with NaOH. The homogenates were then centrifuged at 100 000 $\times$ *g* for 1 h. $NO^{\bullet}$ synthesis was measured by a previously described method [24, 25], in which the oxidation of oxyhaemoglobin to methaemoglobin by $NO^{\bullet}$ is monitored spectrophotometrically. The absorption difference between 401 and 411 nm was continuously monitored with a dual-wavelength recording spectrophotometer by using a bandwidth of 2 nm, at 37 °C.

Statistical calculations

The data resulting from each experimental group were expressed as the mean $\pm$ S.E.M. A Mann–Whitney *U* test *t*-test was used to compare means between the two groups, using SPSS 10.0. Linear regression analysis was applied where indicated. A p value < 0.05 was considered significant.

Results

Obtained from this study iNOS and Na^+K^+ATPase activities in addition to nTyr levels were shown in Table 1. Previously in our study, nTyr levels had been hardly detected in control guinea pig kidneys but nTyr levels had been detected markedly in kidneys of animals exposed to LPS. nTyr levels were also significantly increased in the LPS-injected group when compared to the control (*P*:0.000). As can be seen in Table 1.

The regression analysis between Na^+K^+ATPase activity and nTyr levels of LPS-treated animals revealed negative correlation ($r = -0.868, P = 0.001$) (Fig. 1). Similar to between Na^+K^+ATPase activity with iNOS activity were also negative correlation ($r = -0.877$, $P = 0.001$) (Fig. 2).

Table 1. 3-Nitrotyrosine level, iNOS and Na^+K^+ATPase activities in kidney homogenates of control or exposed guinea pig with *E. coli* lipopolysaccharides

	iNOS (nmol/min/g tissue)	3-nTyr (nmol/g tissue)	Na^+K^+ATPase (μmol P_i/h/mg protein)
Control ($n = 10$)	0.378 $\pm$ 0.019	Hardly detectable	2.604 $\pm$ 0.083
LPS-treatment ($n = 10$)	0.842 $\pm$ 0.044	4.524 $\pm$ 0.255	1.327 $\pm$ 0.078

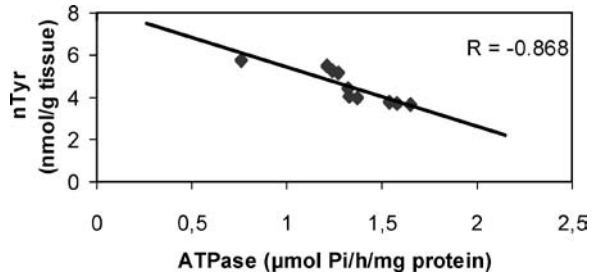

Fig. 1. Correlation between Na^+K^+ATPase activity and 3-nitrotyrosine formation in kidney of guinea pigs exposed to bacterial lipopolysaccharide.

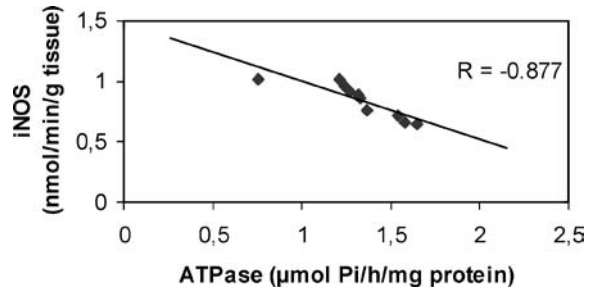

Fig. 2. Correlation between Na^+K^+ATPase activity and iNOS measurements in kidney of guinea pigs exposed to bacterial lipopolysaccharide.

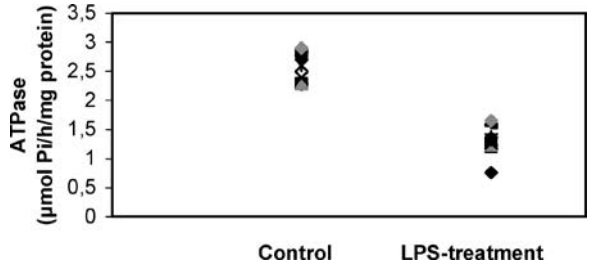

Fig. 3. Na^+K^+ATPase activities in kidneys of guinea pigs exposed to bacterial lipopolysaccharide and control.

In our this study, Na^+K^+ATPase activity significantly decreased in LPS-treated guinea pig kidneys compared to control animals and kidney Na^+K^+ATPase activity were inhibited about 50% after LPS injection (*P*:0.000) (Fig. 3). Although iNOS activities were low level detectable in kidney of control animals, iNOS activity in kidneys of animals exposed to LPS were significantly increased (*P*:0.000) (Fig. 4).

Discussion

Bacterial LPS is present on the outer membranes of all gram-negative bacteria and causes the systemic inflammatory response syndrome and septic shock, which finally develop to multiorgan failure [26]. Septicemia and septic shock are

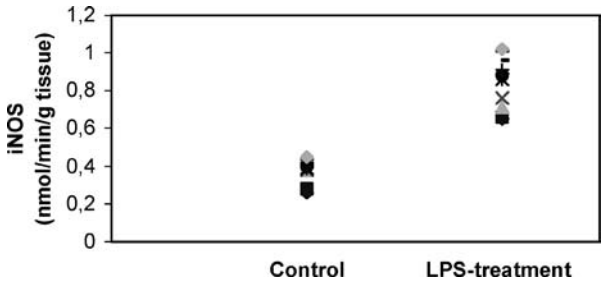

Fig. 4. iNOS measurements in kidneys of guinea pigs exposed to bacterial lipopolysaccharide and control.

associated with high mortality, and current therapy is mostly supportive and largely ineffective. Acute renal failure is a serious complication of septicemia and septic shock. 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 [10].

LPS *via* cytokines can stimulate production of $NO^{\bullet}$ by the activation of the iNOS [4]. Therefore, intraperitoneal LPS treatment up-regulates iNOS and cytokine production in the kidney. That is, when LPS is injected intraperitoneally, iNOS and IL-6 mRNA are induced in the kidney [1]. Cytokine production regulates the host response to inflammation and infection. Although TNF-α and IL-1 are regarded as major mediators of septic shock, IL-1 and to a greater extent TNF-α production may be transitory. TNF-α and IL-1, in turn, induce production of other cytokines such as IL-6. IL-6 levels predict mortality in septic shock and may represent the net effect of biologically active TNF-α and IL-1 [1]. Kidney epithelial cells exposed to cytokines could express an iNOS. Stimulation of kidney epithelial cell with TNF-alpha and IFN-gamma dramatically increase the level of iNOS mRNA [5].

Renal failure is a frequent complication of sepsis with a high mortality. TNF has been suggested to be a factör in the acute renal failure in sepsis or endotoxemia. Recent studies also suggest involvement of $NO^{\bullet}$, generated by iNOS, in the pathogenesis of endotoxin-induced renal failure [27].

LPS treatment *in vivo* induces the expression of an iNOS mRNA and thus provide direct molecular evidence for the involvement of $NO^{\bullet}$ in septic shock [28]. Induction of nitric oxide synthase is critical to the syndrome recognized as sepsis after the administration of bacterial LPS. The sepsis syndrome that followed LPS administration resulted in significant reductions in blood pressure and GFR [4].

In kidney, iNOS activity after LPS treatment was detected in both particulate and soluble fractions. Traditionally, iNOS was considered a soluble enzyme; but recent reports indicate that iNOS is at least partially membrane associated, and iNOS activity in neutrophils isolated from patients with urinary tract infections (UTIs) is primarily particulate [1].

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 $NO^{\bullet}$-mediated oxidant injury to renal proximal tubules [10].

Stimulation of superoxide generation in response to LPS has been suggested in kidney, as has its involvement in organ injury. $NO^{\bullet}$ and superoxide undergo a bi-radical addition reaction to yield $ONOO^{-}$. The rate constant for the reaction of $NO^{\bullet}$ with superoxide (6.7×10^{9} $M^{-1}s^{-1}$) is faster than that for superoxide reacting with superoxide dismutase (SOD) (2.0×10^{9} $M^{-1}s^{-1}$). Thus, the formation of $ONOO^{-}$ is favored in conditions where both $NO^{\bullet}$ and superoxide are formed. $ONOO^{-}$ can react with lipids, proteins, and DNA. $ONOO^{-}$ is difficult to measure *in vivo* because of its short half-life. However, the formation of nTyr-protein adducts is a reliable biomarker of $ONOO^{-}$ generation. The presence of nTyr-protein adducts in the kidney indicated the generation of both $NO^{\bullet}$ and superoxide and the formation of the strong oxidant $ONOO^{-}$ [10].

The aim of this study was to elucidate the effects of LPS on kidney in an attempt to gain a better understanding of the cellular mechanism underlying the pathogenesis of renal dysfunction in endotoxemia and sepsis. For this reason, previously in our study in addition to measure of nTyr levels, in the present study, we investigated the relationship between nitrotyrosine formation with iNOS and Na^{+},K^{+}-ATPase activities in guinea pig kidney after animals received endotoxin for 6 h.

In this study, we have found that there is a significant increase in iNOS activity in kidney following 6 h after administration of *E. coli*-LPS to the animals. This findings is in accord with our previous observations in liver [21], and also consistent with those of others showing that $NO^{\bullet}$ or its metabolites are significantly increased in many organs after LPS administration [28–30].

Liu *et al.* reported that the LPS-induced increase in iNOS mRNA [28] and it has been demonstrated that treatment with LPS caused increase in the amount of iNOS mRNA in many tissues such as in the liver or macrophages isolated from the peritoneum, in glomeruli and the inner medulla, the outer medulla [31]. Furthermore, Mayeux *et al*. demonstrate that proximal tubules express a calcium/calmodulin-dependent NOS activity that is increased *in vivo* by LPS [32]. In addition, Markewitz *et al.* reported that stimulation of kidney epithelial cells with TNF-α and IFN-γ dramatically increased the level of iNOS mRNA [5]. Our findings are also in accordance with those of previous studies on elevated iNOS level in endotoxic or bacteriemic shock.

RNS-induced alterations in organ architecture were shown to associate with organ dysmetabolism or the modification of biochemical markers of organ function, such as enzyme activity [16, 25, 28, 29, 33–37]. In the present study, we demonstrated that excess production of RNS is responsible

for the impaired Na^+,K^+-ATPase activity in inflamed guinea pig kidney. Guzman and co-workers induced $NO^\bullet$ production in mouse proximal tubule epithelial cells by treatment with LPS and IFN-γ followed by determinations of ouabain-sensitive ATPase activity and they reported that Na^+,K^+-ATPase activity decreased after 4 h of LPS/IFN-γ treatment. In addition, Guzman and co-workers reported that $NO^\bullet$ generated by mouse proximal tubule epithelial cell iNOS inhibits Na^+,K^+-ATPase activity in an autocrine fashion and that this inhibition is accompanied by a reduction in Na-dependent solute transport [8]. Our findings are in accordance with those of previous studies on impaired Na^+,K^+-ATPase activity in exposed LPS animals.

In our present study, the negative correlation observed between Na^+,K^+-ATPase and iNOS activity as well as nTyr levels in LPS treated animals strengthens the direct involvement of RNS. These data suggest that $ONOO^-$ signaling participates in the regulation of renal Na^+,K^+-ATPase activity and the endogenous $NO^\bullet$, formation due to elevated iNOS activity, plays a inhibitory role on Na^+,K^+-ATPase activity in the kidney. Furthermore, this finding is also in accord with previous observations in kidney [17, 19]. The results of our current study is not parallel totally with the results of our previous study, showing that Na^+,K^+-ATPase activity of erythrocytes is significantly inhibited when they exposed to $ONOO^-$ *in vitro*, but unchanged in *E. coli* injected rat [16]. The discrepancy may be explained by the difference between the studied tissues and their intracellular antioxidant capacity. Because, it has been demonstrated that the increased erythrocyte antioxidant capacity plays an important protective role against oxidative effects of $ONOO^-$ induced by *E. coli*-LPS [16]. The activity of the transmembrane enzyme Na^+,K^+-ATPase is very susceptible to free radicals and membrane lipid peroxidation [13, 15]. Lipid peroxidation has been shown to alter Na^+,K^+-ATPase function by modification at specific active sites in a selective manner [15]. Depletion of glutathione and other protective antioxidants by RNS may greatly contribute to increasing amount of reactive species, which may also account for impaired activity of Na^+,K^+-ATPase [34]. $ONOO^-$ has been shown to directly oxidize a SH group of the active site of the Na^+,K^+-ATPase and then inhibits this enzyme activity in porcine cerebral cortex [14].

In summary, our results show that although nTyr levels and iNOS activity were increased, Na^+,K^+-ATPase activities were decreased in guinea pig kidney exposed to *E. coli*-LPS. Thus, the negative correlation of Na^+,K^+-ATPase activity was observed with both iNOS activity and nTyr levels in the kidney treated *E. coli*-LPS. In conclusion, the present study have indicated that both endogenous production of $NO^\bullet$ *via* iNOS activity and simultaneously superoxide generation are stimulated in response to LPS. Thus, $NO^\bullet$ and superoxide react spontaneously to form nitrating agent and versatile oxidant $ONOO^-$. Therefore, although Na^+,K^+-ATPase activity is impaired, iNOS activity is increased in response to LPS. In this sense, our this study have demonstrated that RNS-dependent kidney dysfunction also include the modification in membrane Na^+,K^+-ATPase, which impairs the activity of the enzyme. This event may be a crucial component leading to pathological consequences such as kidney dysfunction in which the production of RNS are increased as in the case of LPS challenge.

References

1. Olsson LE, Wheeler MA, Sessa WC, Weiss RM: Bladder instillation and intraperitoneal injection of *Escherichia coli* lipopolysaccharide up-regulate cytokines and iNOS in rat ürinary bladder. J Pharm Exp Ther 284: 1203–1208, 1998
2. Essani NA, McGruice GM, Manning AM, Jaeschke H: Differential induction of mRNA for ICAM-1 and selectins in hepatocytes, kuppfer cells and endothelial cells during endotoxemia. Biochem Biophys Res Commun 221: 74–82, 1995
3. Takahashi M, Ogasawara K, Takeda K, Hashimoto W, Sakihara H, Kumagai K, Anzai R, Satoh M, Seki S: LPS induces NK1.1+alpha beta T cells with potent cytotoxicity in the liver of mice via production of IL-12 from Kuppfer cells. J Immunol 156: 2436–2442, 1996
4. Schwartz D, Mendonca M, Schwartz I, Xia Y, Satriano J, Wilson CB, Blantz RC: Inhibition of constitutive nitric oxide synthase (NOS) by nitric oxide generated by inducible NOS after lipopolysaccharide administration provokes renal dysfunction in rats. J Clin Invest 100: 439–448, 1997
5. Markewitz BA, Michael JR, Kohan DE: Cytokine-induced expression of a nitric oxide synthase in rat renal tubule cells. J Clin Invest 91: 2138–2143, 1993
6. Zurovsky Y, Gispaan I: Antioxidants attenuate endotoxin-induced acute renal failure in rats. Am J Kidney Dis 25: 51–57, 1995
7. Shultz PJ, Raij L: Endogenously synthesized nitric oxide prevents endotoxin-induced glomerular thrombosis. J Clin Invest 90: 1718–1725, 1992
8. Guzman NJ, Fang MZ, Tang SS, Ingelfinger JR, Garg LC: Autocrine inhibition of Na^+K^+ATPase by nitric oxide in mouse proximal tubule epithelial cells. J Clin Invest 95: 2083–2088, 1995
9. Kang YH, Falk MC, Bentley TB, Lee CH: Distriubution and role of lipopolysaccharide in the pathogenesis of acute renal proximal tubule injury. Shock 4: 441–449, 1995
10. Zhang C, Walker LM, Mayeux PR: Role of nitric oxide in lipopolysaccharide-induced oxidant stres in the rat kidney. Biochem Pharmacol 59: 203–209, 2000
11. Fukuyama N, Takebayashi Y, Hida M, Ishida H, Ichimori K, Nakazawa H: Clinical evidence of peroxynitrite formation in chronic renal failure patients with septic shock. Free Radic Biol Med 22: 771–774, 1997
12. Bian K, Davis K, Kuret J, Binder L, Murad F: Nitrotyrosine formation with endotoxin-induced kidney injury detected by immunohistochemistry. Am J Physiol 277: F33–F40, 1999
13. Mishra OP, Delivoria-Papadopoulos M, Cahilane G, Wagerle LC: Lipid peroxidation as the mechanism of modification of the affinity of the Na^+,K^+-ATPase active sites for ATP, K^+,Na^+, and strophanthidin *in vitro*. Neurochem Res 14: 845–851, 1989
14. Sato T, Kamata Y, Irifune M, Nishikawa T: Inhibitory effect of several nitric oxide-generating compounds on purified Na^+,K^+-ATPase activity from porcine cerebral cortex. J Neurochem 68: 1312–1318, 1997
15. Qayyum I, Zubrow AB, Ashraf QM, Kubin J, Delivoria-Papadopoulos M, Mishra OP: Nitration as a mechanism of Na^+,K^+-ATPase

modification during hypoxia in the cerebral cortex of the guinea pig fetus. Neurochem Res 26: 1163–1169, 2001
16. Türközkan N, Ünlü A, Ertabak A, Cimen B, Karabicak U: The effects of peroxynitrite on erythrocytes. Clin Chem Lab Med 39: 1263–1266, 2001
17. Zhang C, Imam SZ, Ali SF, Mayeux PR: Peroxynitrite and the regulation of Na^+,K^+-ATPase activity by angiotensin II in the rat proximal tubule. Nitric Oxide 7: 30–35, 2002
18. Muriel P, Sandoval G: Nitric oxide and peroxynitrite anion modulate liver plasma membrane fluidity and Na^+,K^+-ATPase activity. Nitric Oxide 4: 333–342, 2000
19. Kang DG, Kim JW, Lee J: Effects of nitric oxide synthesis inhibition on the Na,K-ATPase activity in the kidney. Pharm Res 41: 121–125, 2000
20. Unlu A, Türközkan N, Cimen B, Karabiçak U, Yaman H: The effect of *E. coli*-derived lipopolysaccharides on plasma levels of malondialdehyde and 3-nitrotyrosine. Clin Chem Lab Med 39: 491–493, 2001
21. Cimen B, Türközkan N, Seven I, Unlü A, Karasu C: Impaired Na^+K^+ATPase activity as a mechanism of reactive nitrogen species-induced cytotoxicity in guinea pig liver exposed to lipopolysaccharides. Mol Cell Biochem 2004
22. Ertabak A, Kutluay T, Unlu A, Türközkan N, Cimen B, Yaman H: The effects of desferrioxamine on peroxynitrite-induced oxidative damage in erythrocytes. Cell Biochem Funct 2004
23. Serpersu E, Ciliv G: Some properties of (Na^+K^+)-dependent adenosinetriphosphatase from human erythrocytes. Biochem Med 20: 31–39, 1978
24. Feelisch M. Noack EA: Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase. Eur J Pharmacol 139: 19–30, 1987
25. Knowles RG, Marrett M, Salter M, Moncada S: Differential induction of brain, lung and liver nitric oxide synthase by endotoxin in the rat. Biochem J 270: 833–836, 1990
26. Morikawa A, Kato Y, Sugiyama T, Koide N, Chakravortty D, Yoshida T, Yokochi T: Role of nitric oxide in lipopolysaccharide-induced hepatic injury in D-galactosamine-sensitized mice as an experimental endotoxic shock model. Infect Immun 67: 1018–1024, 1999
27. Knotek M, Rogachev B, Wang W, Ecder T, Melnikov V, Gengaro PE, Esson M, Edelstein CL, Dinarello CA, Schrier RW: Endotoxemic renal failure in mice: Role of tumor necrosis factor independent of inducible nitric oxide synthase. Kidney Int 59: 2243–2249, 2001
28. Liu S, Adcock IM, Old RW, Barnes PJ, Evans TW: Lipopolysaccharide treatment *in vivo* induces widespread tissue expression of inducible nitric oxide synthase mRNA. Biochem Biophys Res Commun 196: 1208–1213, 1993
29. Yang F, Comtois AS, Fang L, Hartman NG, Blaise G: Nitric oxide derived-nitrate anion contributes to endotoxic shock and multiple organ injury/dysfunction. Crit Care Med 30: 650–657, 2002
30. Paya D, Stoclet JC: Involvement of bradykinin and nitric oxide in the early hemodynamic effects of lipopolysaccharide in rats. Shock 3: 376–379, 1995
31. Morrissey JJ, McCracken R, Kaneto H, Vehaskari M, Montani D, Klahr S: Location of an inducible nitric oxide synthase mRNA in the normal kidney. Kidney Int 45: 998–1005, 1994
32. Mayeux PR, Garner HR, Gibson JD, Beanum VC: Effect of lipopolysaccharide on nitric oxide synthase activity in rat proximal tubules. Biochem Pharm 49: 115–118, 1995
33. Watanabe N, Miura S, Zeki S, Ishii H: Hepatocellular oxidative DNA injury induced by macrophage-derived nitric oxide. Free Radic Biol Med 30: 1019–1028, 2000
34. D'Ambrossio SM, Gibson-D'Ambrossio RE, Brady T, Oberyszyn AS., Robertson FM: Mechanisms of nitric oxide-induced cytotoxicity in normal human hepatocytes. Environ Mol Mutagen 37: 46–54, 2001
35. Nussler AK, Billiar TR: Inflammation, immunoregulation and inducible nitric oxide synthase. J Leuk Biol 54: 171–178, 1993
36. Sass G, Koerber K, Bang R, Guehring H, Tiegs G: Inducible nitric oxide synthase is critical for immune-mediated liver injury in mice. J Clin Invest 107: 439–447, 2001
37. Kurose I, Miura S, Higuchi H, Watanabe N, Kamegaya Y, Takaishi M, Tomita K, Fukumura D, Kato S, Ishii H: Increased nitric oxide synthase activity as a cause of mitochondrial dysfunction in rat hepatocytes: Roles for tumor necrosis factor-alpha. Hepatology 24: 1185–1192, 1996