Enhanced NO and superoxide generation in dysfunctional hearts from endotoxemic rats

FADI H. KHADOUR,1 DONNA PANAS,1 PÉTER FERDINANDY,2 COSTAS SCHULZE,1 TAMÁS CSONT,1 MANOJ M. LALU,1 STEPHEN M. WILDHIRT,3 AND RICHARD SCHULZ1

1Departments of Pharmacology and Pediatrics, Cardiovascular Research Group, University of Alberta, Edmonton, Alberta T6G 2S2, Canada; 2Department of Biochemistry, Cardiovascular Research Group, University of Szeged, H-6720 Szeged, Hungary; and 3Department of Cardiac Surgery, University Hospital Grosshadern, Ludwig-Maximillians-University, 81377 Munich, Germany

Received 1 March 2001; accepted in final form 6 May 2002

Septic shock is characterized by severe hypotension with profound vasodilatation and multiple organ failure resulting from systemic release of inflammatory cytokines in response to an infective organism (35). Depression of myocardial contractility is a well-documented feature of septic shock (15, 34) despite the fact that assessment of intrinsic cardiac function is complicated by a marked increase in heart rate and decreased preload and afterload. Data from both clinical (32) and experimental (29) studies indicate the presence of genuine myocardial dysfunction when assessed independently of changes in hemodynamics. However, the etiological mechanism(s) of cardiac dysfunction in sepsis is not well understood, but various circulating and/or locally produced mediators have been implicated (for review see Ref. 19).

Evidence from our laboratory (39, 40) and from others (2, 3) suggests that exposure of animal hearts or isolated cardiac myocytes to bacterial endotoxin (lipopolysaccharides, LPS) or proinflammatory cytokines enhanced nitric oxide (NO) generation via induction of NO synthase (iNOS). The production of large amounts of NO by this enzyme may have detrimental effects on the myocardium (2, 9, 39). On the other hand, NO may be cardioprotective (37) and may also act as an antioxidant molecule (45). Recent studies have indicated that many of the deleterious effects of NO are mediated by peroxynitrite; this powerful oxidant is generated from a fast diffusion-limited reaction of NO and superoxide anion (5). It is possible that peroxynitrite contributes to myocardial injury during sepsis if cardiac production of superoxide anion is also enhanced. Indeed, evidence for increased xanthine oxidase activity exists in the gut, liver, spleen, kidney, and lung of mice (26) and in the aorta of rats (10) injected with bacterial LPS. This enzyme is a major source of superoxide production in the rat heart (20). However, the changes in myocardial superoxide biosynthesis, necessary for peroxynitrite generation, are not well characterized in the setting of sepsis.

Peroxynitrite can exert cytotoxic actions by causing lipid peroxidation, nitration of free and protein-associated tyrosine residues, oxidation of sulfhydryl moieties, inhibition of mitochondrial respiration, DNA strand breakage, depletion of intracellular nucleotides via activation of poly ADP-ribose polymerase, and induction of apoptosis (see Refs. 6, 36, 41 for review). In the heart, endogenous formation of peroxynitrite contributes to myocardial stunning in ischemia-reperfu-
Oxygenator (supplied with 95% O2-5% CO2) into the left 0.2% bovine serum albumin (BSA) was delivered from the flow line. Cardiac output and aortic pressure were measured by a Gould P21 pressure transducer in the aortic flow line. The hydrostatic afterload pressure was formed by letting the working heart eject by the working heart into a compliance chamber (containing 1 ml of air) and into C) was ejected by the working heart to form NO-donor nitrosothiols (30, 31). To date, the changes in myocardial peroxynitrite formation during sepsis are unknown.

The objective of this study was to determine the alterations in cardiac mechanical function and efficiency as well as myocardial NO, superoxide, and peroxynitrite generation in a rat model of sepsis induced by in vivo administration of bacterial LPS. The present study was designed to test the hypothesis that cardiac dysfunction in sepsis is accompanied by excessive generation of both NO and superoxide and thus peroxynitrite.

MATERIALS AND METHODS

This study conforms with the “Guide to the Care and Use of Experimental Animals” published by the Canadian Council on Animal Care (Revised 1993).

Experimental design. Male Sprague-Dawley rats (275–350 g) were injected intraperitoneally either with 4 mg/kg LPS (Salmonella typhosa 0901, Difco) or pyrogen-free saline vehicle (control). Six hours later, animals were anesthetized with pentobarbital sodium (50 mg/kg ip), and hearts were rapidly excised. Hearts were either perfused in the working mode for assessment of their mechanical function and efficiency or perfused for 10 min in the Langendorff mode to wash them free of blood, and they were then frozen in liquid nitrogen and stored at −80°C for subsequent biochemical analysis.

Hearts perfusions. Experiments with the isolated working rat hearts were performed as previously described (17). Briefly, hearts were cannulated via the aorta and were initially perfused in a retrograde manner (Langendorff method) with Krebs-Henseleit buffer at 37°C for 10 min. During this period, the left atrium and pulmonary artery were cannulated, and the perfusion was switched to the working heart mode. The working heart perfuse [recirculating Krebs-Henseleit solution containing 11 mM glucose, 5 mM pyruvate, 100 μU/ml insulin, 1.75 mM Ca2+, 0.5 mM EDTA, and 0.2% bovine serum albumin (BSA)] was delivered from the oxygenator (supplied with 95% O2-5% CO2) into the left atrium at a hydrostatic preload equivalent to 9.5 mm Hg. The perfusate (pH 7.4, 37°C) was ejected by the working heart into a compliance chamber (containing 1 ml of air) and into the aortic outflow line. The hydrostatic afterload pressure was set at a column height equivalent to 70 mm Hg. Hearts were paced at 300 beats/min throughout the experiment with a Grass SD9 stimulator (regular stimuli; 0.6 ms duration; 0.4 ms delay) with leads placed on the aortic and atrial cannulas. Heart rate and peak systolic pressure were measured by a Gould P21 pressure transducer in the aortic outflow line. Cardiac output and aortic flow were measured by using Transonic flow probes in the preload and afterload lines, respectively. Coronary flow was calculated as the difference between cardiac output and aortic flow. Myocardial O2 consumption was calculated on the basis of coronary flow rates, and the difference between left atrial and pulmonary artery O2 contents was measured using micro-O2 electrodes (Yellow Springs Instruments; Yellow Springs, OH). After 20 min of equilibration in the working mode, cardiac output, aortic pressure, and coronary flow were recorded continuously for 60 min. Cardiac work, the product of cardiac output (ml/min) times peak systolic pressure (mmHg), was used as an index of mechanical function.

Blood sampling and measurements of plasma nitrate/nitrite. One milliliter of blood was collected from the thoracic cavity with a heparinized syringe immediately after removal of the heart, and the plasma was obtained by centrifugation at 2,000 g for 5 min at 4°C and stored at −20°C until analysis. After thawing was completed, plasma was diluted (1:1 vol/vol) with deionized water. Then 400 μl of the diluted plasma were deproteinized by centrifugal ultrafiltration (Millipore Ultrafree-MC microcentrifuge tubes UFC3; Bedford, MA). Ultrafiltrates were analyzed for total nitrate and nitrite content by the method of Green et al. (18). The limit of detection was 0.1 μM for both nitrate and nitrite.

Measurement of ventricular NO content. Total ventricular NO content was measured using electron spin resonance (ESR) spectroscopy after ex vivo spin trapping of NO as previously described (11). Briefly, ~100 mg of both ventricles were removed, minced to pieces of ~3 mm in size, immersed into the spin-trapping solution [55 mM N-methyl-glucosamine-dithiocarbamate (MGD) and 12 mM FeSO4 dissolved in distilled water, pH set to 7.4 with HCl], and incubated at 5 min at 20°C. Samples were then transferred into quartz tubes and quickly frozen in liquid nitrogen and then stored at −80°C. ESR spectra of the NO-Fe2+ (MGD)2 complex were then measured at a temperature of 80 K with a Bruker ESP300 spectrometer equipped with an Oxford Instruments ESR-900 flowing helium cryostat (ESR parameters: X-band, 100 kHz modulation frequency; 10 mW microwave power), and the NO signals were evaluated as described (11).

Determination of ventricular NOS activities. The frozen ventricular tissues were prepared for the assay of NOS activity as previously described (24). A portion of the powdered ventricular tissue was homogenized and centrifuged (1,000 g, 5 min, 4°C) to obtain the whole cell homogenate. The resultant supernatant was used for measurement of ventricular NOS activities by the conversion of L-[14C]arginine to [14C]citrulline. NOS activity was determined as the rate of citrulline production using a biochemical assay utilizing BSA as a standard (expressed as pmol·min−1·mg protein−1). The limit of detection was 0.1 pmol·min−1·mg protein−1.

Measurement of ventricular superoxide content. Ventricular myocardial superoxide content was determined by lucigenin-enhanced luminescence as described previously (12, 16, 47) with some modification. Approximately 100 mg of both ventricles were cut off, immediately minced, and then incubated in 5 ml of air-equilibrated Krebs-Henseleit solution containing 10 mM HEPES-NaOH (pH 7.4) with or without Nω-monomethyl-l-arginine (l-NMMA) (1 mM) for 20 min at room temperature. The samples were then placed into glass tubes containing 10 μM lucigenin in a final volume of 1 ml Krebs-Henseleit solution. The chemiluminescence of the sample was measured in a luminometer (Berthold Lumat LB9501) as relative light units (RLU) emitted, integrated over 30-s intervals for 5 min. The last 2.5 min were used for the reading, a time when the signal plateaued. The vial containing the buffer and lucigenin was read first to determine background luminescence, then ventricular tissue was added, and luminescence was determined again. Superoxide levels were reported as RLU after background luminescence subtraction and were normalized to milligram wet tissue weight.
To validate this method, in some experiments the superoxide scavenger nitro blue tetrazolium (300 μM) or the superoxide dismutase (SOD) mimic manganese(III) tetrakis (N-ethylpyridinium-2-y)porphyrin (50 μM) (4) was added to vials following luminescence measurements in the presence of cardiac tissue. Both of these structurally distinct SOD mimetic compounds decreased luminescence signal to background levels. In additional experiments the effect of a xenanthine oxidase inhibitor allopurinol was investigated on cardiac superoxide production in LPS-treated rats. Minced ventricular tissue was incubated for 15 min at room temperature with 50 μM allopurinol in the presence or absence of L-NMMA.

**Determination of ventricular xanthine oxidoreductase activities.** Samples of powdered ventricular tissue (crushed in a mortar and pestle under liquid nitrogen) were homogenized and centrifuged (100,000 g, 35 min, 4°C) to obtain the cytosolic fraction. The cytosolic fraction was used to determine the activities of xanthine oxidase and its precursor xanthine dehydrogenase as previously described (7, 16, 17). This procedure was based on the conversion of pterine to isoxanthopterine, a fluorometric product, in the presence and absence of the electron acceptor methylene blue. Xanthine oxidoreductase activities were determined as the rate of isoxanthopterine production, which was normalized to the protein concentration of the homogenates, as determined by bicinchoninic acid assay utilizing BSA as a standard (expressed as pmol × min⁻¹ × mg protein⁻¹).

**Immunohistochemistry for ventricular inducible NOS and nitrotyrosine.** Control and LPS hearts (n = 4 in each group) were isolated and perfused with Krebs-Henseleit buffer in Langendorff mode at a constant pressure of 70 mmHg for 30 min. Hearts were then cross-sectioned (2–3 mm thickness), embedded in a special medium (Histo-Prep, Fisher Scientific), frozen in liquid nitrogen, and then subsequently stored at −70°C. Later, adjacent cryosections (3 μm thickness) were obtained and used for detection of inducible NO synthase (iNOS) and nitrotyrosine-containing proteins. Sections were fixed with chloroform (30 min) and blocked with 1.5% H₂O₂ (10 min). After washing (twice, 5 min each) was completed, nonspecific binding was blocked with Protein Block serum-free reagent (Dako). For detection of iNOS, sections were incubated with monoclonal mouse anti-human antibodies at 1:100 dilution (Transduction Laboratories; Lexington, KY) for 45 min and then washed (three times, 5 min each), and a biotinylated antibody was applied for 30 min. Subsequently, sections were treated with peroxidase-conjugated streptavidin for 30 min. For detection of nitrotyrosine, sections were incubated with polyclonal antibodies (purified rabbit IgG, Upstate/Biozol) for 45 min at 1:300 dilution and then washed (three times, 5 min each), and secondary antibodies (Dako Envision system) were applied for 30 min. All sections were visualized with a peroxidase substrate AEC kit (Vector). After rinsing was completed, sections were counterstained with Mayer’s hemalum solution and mounted. All steps were performed at room temperature. Negative controls were obtained by omitting the primary antibody. Isotype controls for iNOS were obtained using a mouse IgG1 (1:400 dilution, Sigma) instead of the first antibody.

**Measurements of ventricular lipid hydroperoxides.** The level of lipid hydroperoxides, a marker of oxidative injury to tissue, was measured in the powdered ventricular tissue by a colorimetric assay according to the manufacturer’s instructions (Cayman Chemical; Ann Arbor, MI). This assay is based on the redox reaction of lipid hydroperoxides with Fe²⁺. The resulting Fe³⁺ is detected using thioate anion as the chromogen. Lipid hydroperoxides were first quantitatively extracted from ventricular samples (50–60 mg) by a chloroform-methanol mixture (2:1, vol/vol), and then the level of lipid hydroperoxides was determined in the chloroform extract of the samples by using a standard curve (expressed per mg wet weight of ventricular tissue).

**Materials.** The following materials were purchased from the following suppliers: BSA (fraction V), Boehringer Mannheim; l-[U-¹⁴C]arginine monohydrochloride, Amersham; and l-NMMA acetate, Alexis. All other reagents were obtained from Sigma or VWR Canlab.

**Statistical analyses.** Data are presented as means ± SE of samples from n separate experimental animals. Student’s t-test (paired or unpaired) or two-way repeated-measure ANOVA was used to evaluate differences between the groups, as appropriate. Differences were considered significant at P < 0.05. Data analysis was done using a statistical software package (SPSS, version 10.0.5, SPSS; Chicago, IL).

**RESULTS**

**Cardiac mechanical function and efficiency.** Figure 1 depicts cardiac performance and O₂ consumption in the two groups of hearts over the 60-min perfusion period. At the beginning of perfusion, cardiac work in the control group was ~20,000 compared with 2,800 ml × min⁻¹ × mmHg × g dry wt⁻¹ in LPS-treated hearts.

---

**Fig. 1.** Time course of cardiac work (A), O₂ consumption rate (B), and cardiac efficiency (C) in isolated working hearts from control (n = 18) and lipopolysaccharide (LPS)-injected (n = 34) rats, normalized to grams of dry weight of ventricular tissue. Cardiac work = cardiac output × peak systolic pressure; cardiac efficiency = cardiac work/O₂ consumption rate. Differences between the groups were significant by two-way repeated measure ANOVA.

*AjP-Heart Circ Physiol • VOL 283 • SEPTEMBER 2002 • www.ajpheart.org*
Cardiac work remained constant in both groups throughout the perfusion period with the marked differences in their performance persisting until the end of perfusion (Fig. 1A). Similarly, the consumption of O2 by the two groups of hearts was constant during the perfusion period but was significantly depressed in LPS-treated hearts to only 40% of that in control hearts (Fig. 1B). As a result of a proportionately greater decline in cardiac work than that in O2 consumption, cardiac efficiency was markedly depressed in LPS hearts compared with 30% of control values (Fig. 1C).

**Plasma nitrate/nitrite, ventricular NO content, and NOS activity.** The alterations in plasma nitrate/nitrite level (a stable marker of endogenous NO production), ventricular NO content, and NOS activity by LPS treatment are shown in Fig. 2. The level of plasma nitrate/nitrite level was markedly elevated in endotoxemic rats and was ~20-fold higher than that in control animals (Fig. 2A). In addition, the ventricular ESR signal intensity of NO was almost double in LPS-treated hearts compared with control hearts (Fig. 2B). The Ca2+-independent NOS activity (iNOS) in ventricular homogenates from control animals was at the lower limit of detection and was markedly increased (20-fold) in LPS-treated hearts (Fig. 2C). Ca2+-dependent NOS activity was not significantly altered by LPS treatment (0.9 ± 0.2 vs. 0.9 ± 0.3 pmol × min−1 × mg protein−1 in control and LPS-treated hearts, respectively).

**Ventricular superoxide content and xanthine oxidoreductase activities.** Figure 3 shows the changes in ventricular superoxide and xanthine oxidoreductase activities as a result of LPS treatment. Myocardial superoxide production in control hearts was ~1.75 RLU/mg wet wt of ventricular tissues and was significantly increased in LPS-treated hearts (Fig. 3A). When tissues from both groups were incubated with the NOS inhibitor L-NMMA, the superoxide-dependent chemiluminescence was enhanced in both groups but remained significantly higher in LPS-treated hearts compared with controls. The xanthine oxidase inhibitor allopurinol partially reduced the chemiluminescence signal in hearts from LPS-treated rats, both in the absence (by 23.7 ± 7.1%, n = 4, P = 0.054) or presence of L-NMMA (by 17.3 ± 7.7%, n = 4, P = 0.054). The activity of xanthine dehydrogenase, a precursor of xanthine oxidase, was increased by ~55% in LPS-treated hearts compared with that of control hearts (Fig. 3B). Furthermore, xanthine oxidase activ-

---

**Fig. 2.** Plasma nitrate/nitrite level (A), ventricular nitric oxide (NO) content (B), and Ca2+-independent NO synthase (NOS) activity (C) in hearts from control (open bars) and LPS-injected (filled bars) rats. Number of samples per group in parenthesis. *P < 0.05 vs. control (unpaired t-test).

**Fig. 3.** Ventricular superoxide content (A) and xanthine oxidoreductase activities (B) in hearts from control and LPS-injected rats. L-NMMA, Nω-monomethyl-L-arginine; RLU, relative light units; n = 5–7 in each group. *P < 0.05 (unpaired t-test).
ity, a source of superoxide production in the myocardium, was significantly elevated by LPS treatment and was double that of controls (Fig. 3B).

**Ventricular iNOS, nitrotyrosine, and hydroperoxides levels.** iNOS immunoreactivity was not detected in control hearts but was expressed in LPS-treated hearts (Fig. 4). The signal was detectable in both microvascular endothelial cells and in some cardiomyocytes of LPS-treated hearts. In control hearts, staining for nitrotyrosine was nearly absent. In contrast, there was abundant nitrotyrosine immunostaining in LPS-treated rat hearts, primarily localized within endothelial cells throughout the myocardium. Negative controls for iNOS and nitrotyrosine did not show any staining (data not shown). LPS treatment more than doubled the level of lipid hydroperoxides in the ventricles (123.7 ± 20.1 vs. 61.7 ± 6.7 nmol × mg wet wt tissue$^{-1}$ in controls, $P < 0.05$).

**DISCUSSION**

The present study for the first time demonstrates increased superoxide generation in the myocardium of endotoxemic rats concurrent with increased myocardial NO production. In addition, this study provides evidence for enhanced myocardial generation of peroxynitrite and oxidative stress injury in hearts from LPS-injected rats. These alterations in the generation of nitroxy-free radicals in LPS-treated hearts were associated with impaired cardiac contractility and depressed cardiac efficiency.

The mechanical function of septic hearts was markedly impaired compared with controls, although in both groups it remained stable over 60 min of perfusion. These data confirm previous reports of myocardial dysfunction during sepsis (15, 29, 32, 34). Oxygen consumption was also impaired in septic hearts, although to a lesser extent than cardiac work. As a result, cardiac efficiency was depressed. The mechanism of cardiac dysfunction during sepsis is not well understood, but several hypotheses have been proposed (19).

The current study shows increased ventricular NO content, iNOS activity, and protein expression in LPS-treated hearts along with an elevated level of plasma nitrate/nitrite. These findings confirm previous data from our group and from others that bacterial endotoxin and proinflammatory cytokines stimulate the expression of iNOS in isolated cardiac myocytes and rat heart (2, 3, 16, 39, 40). This enhanced production of NO reportedly contributes to the depression of myocardial contractility (2, 3, 9, 33). However, selective iNOS inhibitors only partially reversed myocardial dysfunction in rats exposed to endotoxin in vivo (21) or in isolated working rat hearts exposed to proinflammatory cytokines in vitro (33). Therefore, other mechanism(s) or factor(s) may be responsible for cardiac dysfunction during sepsis, apart from excess NO.

Our present investigation is the first to report increased myocardial superoxide content, as measured directly by chemiluminescence, in a rat model of sepsis. In addition, we also showed enhanced myocardial activities of xanthine oxidase and its precursor enzyme xanthine dehydrogenase. The xanthine oxidoreductase system is one of the major sources of superoxide production in the normal rat heart (20); other possible sources include NAD(P)H oxidoreductases (28), mitochondrial respiratory enzymes, and even NO itself when levels of either L-arginine or tetrahydrobiopterin are reduced (see Ref. 1 for review). Our finding that myocardial superoxide generation is partially inhibited

![Fig. 4. Representative myocardial sections from control (A, B) and LPS-injected rats (C–F) stained for inducible NO (iNOS) (A, C, and E) or nitrotyrosine (B, D, and F) by immunohistochemistry. iNOS was not expressed in control hearts (A), but there was abundant specific iNOS immunoreactivity (red staining) in LPS hearts (C). High-power magnification shows that the iNOS signal is present in both microvascular endothelial cells and some cardiomyocytes of LPS-treated hearts (E). Nitrotyrosine staining (red staining) was almost absent in control hearts (B) but was expressed in LPS-treated hearts (D). Nitrotyrosine signal was primarily detected in endothelial cells throughout the myocardium of LPS-treated hearts as revealed by high-power magnification (F). Magnification is ×200 in A–D and ×400 in E and F.](http://ajpheart.physiology.org/10.2233/ajpheart.02098)
by the xanthine oxidase inhibitor allopurinol supports the fact that other enzymatic or nonenzymatic mechanisms for superoxide production exist in septic hearts, in addition to xanthine oxidoreductase. That L-NMMA was not able to block superoxide generation suggests that NOS itself was not a source of this free radical.

These results are in agreement with a recent study from our laboratory of increased myocardial superoxide and xanthine oxidase activity in isolated working rat hearts challenged with proinflammatory cytokines (16). In support of our findings, Brandes et al. (10) recently showed an increased superoxide formation via increased expression of xanthine oxidase and NAD(P)H oxidase in rat aorta challenged with LPS. Therefore, a possible mechanism for the loss of cardiac inotropism during endotoxin challenge may be an endotoxin-induced and cytokine-mediated generation of reactive oxygen-free species such as superoxide. This together with increased NO production that reacts readily with superoxide in a diffusion rate-limited reaction \( k = 6.7 \times 10^9 \text{M}^{-1}\text{s}^{-1} \) (5) argues for enhanced myocardial generation of peroxynitrite during sepsis.

Peroxynitrite, but not NO, reacts with the phenolic ring of tyrosine to form a stable product, 3-nitro-L-tyrosine. Nitrotyrosine is widely used as a marker for the detection of peroxynitrite formation in vivo (8, 25). Some reports (13, 14) have questioned the specificity of this marker for peroxynitrite. For example, these studies have shown in vitro that the nitration of tyrosine occurs in the presence of nitrite and HOCl. HOCl can be formed by the action of neutrophil myeloperoxidase on \( \text{H}_2\text{O}_2 \). Whether this mechanism has a significant contribution to nitrotyrosine formation in intact tissue under different physiological and pathophysiological conditions remains unclear. In addition, nitrotyrosine levels in the brain infarct region of mice subjected to cerebral ischemia-reperfusion remained significantly increased in myeloperoxidase knockout mice compared with wild-type controls (42). This suggests a minor role for the myeloperoxidase/nitrite-dependent nitrotyrosine formation in vivo. Furthermore, Whiteham and Halliwell (44) reported a rapid loss of 3-nitrotyrosine upon its exposure to HOCl, suggesting that the myeloperoxidase pathway may decrease rather than increase nitrotyrosine in tissues where both peroxynitrite and myeloperoxidase exist. On the other hand, we have recently shown that in isolated working rat hearts treated with proinflammatory cytokines (a model in which both nitrite and myeloperoxidase activity are very low), the level of nitrotyrosine increased in the perfusate. Moreover, a peroxynitrite decomposition catalyst prevented the increase in nitrotyrosine and the impairment in myocardial mechanical function (16). These studies, therefore, suggest that nitrotyrosine is indeed a useful estimate of tissue peroxynitrite formation.

In the present investigation the nitrotyrosine signal was increased in LPS-treated hearts, suggesting increased peroxynitrite generation in the myocardium during endotoxemia. Relevantly, Kamisaki et al. (23) reported a prolonged increase in plasma nitrotyrosine levels in rats injected with LPS, an effect that was dose dependent. Although our study did not address specifically the cause-and-effect relationship between peroxynitrite and cardiac dysfunction in sepsis, we have previously shown that infusion of authentic peroxynitrite into the isolated working rat heart induced a delayed (after 30–40 min) depression of cardiac mechanical function with impaired efficiency (48). Furthermore, peroxynitrite is known to inhibit mitochondrial respiration, contractile function of cardiac myocytes, and the isolated left ventricular papillary muscle (22, 46). Therefore, the generation of peroxynitrite by the septic heart may contribute to its dysfunction. Accordingly the use of a peroxynitrite scavenger may protect the heart against sepsis-induced contractile dysfunction.

We have shown by immunohistochemistry that iNOS was localized in both cardiac myocytes and the microvascular endothelium of LPS-treated rat hearts, whereas nitrotyrosine staining was primarily localized in the endothelium. Both sites may potentially contribute to peroxynitrite formation in the heart. It is unclear whether the effects of peroxynitrite are exerted locally or in a paracrine fashion. To date, the specific mechanism of peroxynitrite-induced contractile dysfunction is unknown, but one possible contribution is lipid peroxidation. We also found an increased level of lipid hydroperoxides in LPS-treated hearts compared with controls. However, peroxynitrite has several other cytotoxic effects beside nitration of tyrosine residues and lipid peroxidation that may account for its detrimental actions (6, 36, 41). For example, myocardial matrix metalloproteinases are activated in hearts subjected to the infusion of peroxynitrite, and the resultant loss of mechanical function was inhibited either by the antioxidant glutathione or by an inhibitor of matrix metalloproteinases (43).

In conclusion, our results show for the first time a simultaneous increase in both NO and superoxide generation in the myocardium of hearts from endotoxemic rats. The resultant increased peroxynitrite generation and lipid peroxidation was accompanied by impaired cardiac function. Further studies are required to implicate peroxynitrite in the etiology of sepsis-induced cardiac dysfunction and to delineate the specific mechanism of peroxynitrite-mediated myocardial injury.

We thank Nicole Conrad for help with the immunohistochemical analysis and Jolanta Sawicka for help with the lucigenin chemiluminescence.

This work was supported by a grant provided by the Canadian Institutes for Health Research (MT-11563). R. Schulz is a senior scholar of the Alberta Heritage Foundation for Medical Research. Support for students and postdoctoral fellows was provided by the Alberta Heritage Foundation for Medical Research (to F. Khadour, P. Ferdinandy, T. Csont, and M. M. Lalu), the Heart and Stroke Foundation of Canada (to F. Khadour and C. Schulze), and the Canadian Institutes for Health Research (to P. Ferdinandy).

REFERENCES
2. Balligand JL, Ungureanu-Longrois D, Kelly RA, Kobzik L, Pimental D, Michel T, and Smith TW. Abnormal contractile...


5. Beckman JS, Beckman TW, Chen J, Marshall PA, and Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 87: 1620–1624, 1990.


AJp-Heart Circ Physiol • Vol. 283 • September 2002 • www.ajpheart.org


