Activation of Cardiac Endothelium as a Compensatory Component in Endotoxin-Induced Cardiomyopathy
Role of Endothelin, Prostaglandins, and Nitric Oxide

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Background—In view of growing evidence of an important endothelial paracrine regulation of cardiac function, the present study investigated the role of cardiac endothelium-derived endothelin-1 (ET-1), prostaglandins, and nitric oxide (NO) during endotoxin-induced cardiomyopathy in rabbits.

Methods and Results—Immunohistochemical studies showed a marked transient coinduction of the inducible isoforms of NO synthase (NOS-2) and cyclooxygenase (COX-2) in endocardial endothelium and coronary arteriolar endothelium of hearts 12 hours after intravenous administration of lipopolysaccharide (LPS/H110012h); staining for both isoforms was much weaker 24 hours later (LPS/H1100136h). Nitrotyrosine localization was similar to that of NOS-2, suggesting a NOS-2–related endothelial formation of peroxynitrite in septic hearts. Contractile performance of papillary muscles was depressed in both LPS-treated groups. In the LPS/H1100112h group, however, isometric twitches were significantly prolonged (482±14 versus 420±14 ms in the saline-treated group, P<0.005). This twitch prolongation was completely reversed by simultaneous administration of BQ-123 and indomethacin to block endogenous ET-1 and prostaglandins, respectively. In addition, in the LPS/H1100112h group, myocardial inotropic responsiveness to exogenous ET-1 was enhanced (P<0.01).

Conclusions—Cardiac endothelial activation and myocardial sensitization to endothelium-derived mediators may be part of an adaptive response in the early (12 hours) stages of septic cardiomyopathy. (Circulation. 2001;104:3137-3144.)

Key Words: prostaglandins ■ endocardium ■ myocardium ■ nitric oxide synthase ■ infection

There is growing experimental evidence for a paracrine regulation of cardiac performance by cardiac endothelial cells that is analogous to vascular endothelial control of vascular tone (for review see Bruttsaert et al1 and Shah and MacCarthy2,3). Endocardial and coronary endothelium release diffusible factors, such as nitric oxide (NO), prostaglandins (PGs), and endothelin-1 (ET-1). During sepsis and states of activated inflammation, the endothelium in many vascular beds becomes activated or dysfunctional (for review see Volk and Kox4). The function of cardiac endothelial cells in sepsis, however, is little studied, and it remains unknown whether and how paracrine endothelial factors influence myocardial function in states of systemic inflammation and septic shock.

During sepsis, cardiac performance is impaired.5 Typically, several measures of cardiac performance, eg, ejection fraction and Emax, are decreased. Although sepsis-induced impairment in cardiac performance appears to be related more to myocardial dysfunction than to load abnormalities,6 the underlying mechanisms are not fully understood.

Accordingly, the aim of the present study was to investigate the function of cardiac endothelium in a rabbit model of endotoxicemia-associated cardiac depression. The study was performed in conscious animals with moderate hypotension, minor metabolic changes, and low mortality. The role of the cardiac endothelium in endotoxin-induced cardiomyopathy was assessed by biochemical, immunohistochemical, and physiological analysis, ie, by examining expression and/or activity of inducible NO synthase (NOS-2) and cyclooxygenase (COX-2), endogenous activity of NO, peroxynitrite, PGs, and ET-1, and myocardial responsiveness to exogenous ET-1.

Methods

Rabbits

Experiments were carried out in conscious male New Zealand White rabbits (2 to 2.5 kg) in accordance with approved guidelines for care and use of animals in our institution.
Experimental Protocol
Conscious rabbits were randomized and received an intravenous injection (1.2 mL) via a marginal ear vein: either sterile 0.9% NaCl solution (saline; control group) or endotoxin suspension (600 μg/kg; a mixture of equal amount of 3 types of bacterial lipopolysaccharide [LPS]: Escherichia coli, Salmonella enteritidis, and Salmonella minnesota). Rabbits euthanized randomly at 6, 12, or 36 hours after the administration of pharmacological agents, except for the negative controls (ie, without primary antibody). Was considered positive when it exceeded the background level of 3.3 mol/L atenolol and 0.1 mmol/L glutathione to prevent depression was verified by incubating muscles either with superoxide receptor antagonist). In separate experiments without glutathione, the ET receptor antagonist) was studied by incubating muscles either (1) simultaneously or (2) separately with ET, was studied by incubating muscles either (1) simultaneously or (2) separately with ET, was studied by incubating muscles either (1) simultaneously or (2) separately with ET.

Preparation for Microscopy
For en face confocal microscopy, strips of endocardial tissue were dissected and rinsed in PBS. For cryosections, heart tissue was immersed in a 30% sucrose solution before storage in liquid nitrogen. Tissue strips and cryostat sections were stained with fluorochromes to label F-actin and DNA/RNA with FITC-phalloidin (3.3 μmol/L, Sigma) and propidium iodide (150 μmol/L, Molecular Probes), respectively, or with immunofluorescence to label NOS-2, COX-2 (Transduction Laboratories), 3-nitrotyrosine (tissue marker of peroxynitrite production, UBI), or ET-1 (Biosciences). Staining was considered positive when it exceeded the background level of the negative controls (ie, without primary antibody).

Isolated Papillary Muscle Studies
Papillary muscles (n=53) were mounted vertically in an organ bath filled with Krebs-Ringer solution as described. All buffer solutions contained 20 μmol/L atenolol and 0.1 mmol/L glutathione to prevent β-adrenergic and reactive oxygen species-mediated effects. Measurements were derived from isometric twitches at Lmax, ie, muscle length for maximal active isometric twitch tension; peak active isometric twitch tension (AT); peak rate of isometric tension development (+dT/dt); and the time from stimulus to 50% decline of isometric tension (time to half relaxation, tHR). Force measurements were normalized for muscle cross-sectional area, calculated by dividing the wet weight by Lmax.

Study A
After the stabilization period, baseline performance was measured. The contribution of endogenous mediators, such as NO, PGs, and ET, was studied by incubating muscles either (1) simultaneously or (2) separately with Nω-monomethyl-L-arginine (L-NMMA, 100 μmol/L, Sigma; an NOS blocker), indomethacin (10 μmol/L, a COX inhibitor), and BQ-123 (1 μmol/L, Sigma; a competitive ET1 receptor antagonist). In separate experiments without glutathione, the contribution of endogenous NO to endotoxin-induced myocardial depression was verified by incubating muscles either with superoxide dismutase (SOD, 100 U/mL), L-NMMA (100 and 500 μmol/L), or an excess of L-arginine (1 mmol/L, Sigma).

Study B
After the stabilization period, responses to cumulative concentrations of exogenous ET-1 (from 0.1 nmol/L to 0.1 μmol/L, Sigma) were obtained. During both protocols, isometric twitches were measured 20 minutes after the administration of pharmacological agents, except for BQ-123, in which measurements were obtained twice, ie, after 30 and 60 minutes.

Inducible NOS Activity
NOS activity was measured by conversion of [14C]-arginine to [14C]-citrulline in the absence of calcium and calmodulin to selectively assess the activity of NOS-2.

Endothelin Receptor Properties
For binding experiments, membranes (20 μg protein/mL) were incubated with 250 pmol/L [3H]BQ-123 (42.7 Ci/mmol) and various concentrations of unlabeled ET-1 (Nsysystems). Data were fitted by use of LIGAND software (Jandel Scientifics) to yield maximum binding density and Ki values of ET1/ETα receptor complexes. A Ki value of BQ-123 receptor complexes of 1 nmol/L, determined in parallel experiments, was used in all calculations.

Statistics
Results are expressed as mean±SEM. When appropriate, statistical comparisons of 2 groups of data were made with a Student’s t test. LPS- and saline-treated animals were compared by 2-way ANOVA with repeated measures for in vivo experiments. Comparison among groups of animals (control, LPS 6h, LPS 12h, and LPS 36h) was made by 1-way ANOVA (multiple comparisons according to Dunnett) for ex vivo mechanical studies, including the area under the curve (Simpson’s rule) of ET1 dose-response curves.

Results
Hemodynamics and Biological Parameters In Vivo
The mortality rate after LPS injection was 9%. LPS induced a moderate hypotension, consistently increased body temperature, and decreased body weight (Table 1). Arterial blood gases and ionic composition, however, were similar between the saline and LPS-treated groups (data not shown). Plasma ET1 concentration was increased in LPS-treated rabbits (P<0.01).

Histology and Immunohistochemistry
Histological analysis of cardiac endothelium showed absence of myocardial edema after LPS administration (Figure 1, C through F). Twelve hours after LPS injection, nuclei of endocardial endothelial cells were less intensely stained and the rim of juxtanuclear cytoplasmic RNA was much larger than in endocardial endothelium from control rabbits (Figure 1, A through D). These alterations were also observed in vascular endothelium from coronary artery but not from coronary vein (Figure 1, E and F). In addition, inflammatory cells appeared in both subendocardial and perivascular spaces in the LPS+12h hearts (Figure 1D and 3B). Similar histological alterations were noted in cardiac endothelium in the LPS+36h group (data not shown).

Immunohistochemical studies showed negative staining for NOS-2 and COX-2 in control and LPS+6h hearts (data not shown). Both NOS-2 and COX-2, however, were induced in hearts in the LPS+12h group (Figures 2 and 3). NOS-2 labeling was intensely present in endothelial cells from endocardial endothelium and from coronary arterioles, as well as in inflammatory cells (Figures 2, A and B, and 3, A through C). By contrast, NOS-2 labeling was much weaker in ventricular myocytes, coronary smooth muscle cells, and endothelial cells from coronary veins (Figure 3, A through D). Similarly, COX-2 labeling was more intense in endothelial cells from endocardial endothelium and from coronary arterioles than in ventricular myocytes and coronary smooth muscle cells (Figures 2, E and F, and 3, E and F). Importantly,
en face confocal microscopy revealed a clearly more intense staining for both NOS-2 and COX-2 in endothelial cells in LPS/H11001 12h than in LPS/H11001 36h (Figure 2) hearts. Furthermore, the localization of nitrotyrosine was similar to that in NOS-2 in cardiac endothelial cells of LPS/H11001 12h hearts (Figure 4). In addition, ET-1 staining was quasi-exclusively located in endothelial cells in hearts from control and LPS-treated animals, with no difference in the intensity of staining among studied hearts (data not shown).

**Mechanical Studies**

The baseline performance of papillary muscles isolated from animals exposed to LPS was markedly depressed (Table 2). AT and +dT/dt were dramatically depressed in

**Table 1. Animal Characteristics Recorded Before and 12 or 36 Hours After Saline (n=6) or LPS (n=6) Treatment in Conscious, Chronically Instrumented Rabbits**

<table>
<thead>
<tr>
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<td>Heart rate, bpm</td>
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<tr>
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P values are for interaction between saline-LPS and time after treatment.

**Figure 1.** Dual-channel en face confocal images of endothelium stained with FITC-phalloidin and propidium iodide. Green represents F-actin; red, nuclei and cytoplasmic RNA. A and B, In endocardial endothelium (EE) of control rabbits, F-actin is restricted to periphery; few stress fibers are present in central part of cell. Staining with propidium iodide ranges from nearly selective labeling of nuclei (A) to labeling of nuclei and a thin rim of juxtanuclear cytoplasm (B, white arrows), sometimes negatively outlining Golgi bodies (B, yellow arrows). A, Arrows indicate nuclei of subendothelial interstitial cells. C and D, 12 hours after induction of sepsis, nuclei are less intensely stained and rim of cytoplasmic RNA staining is much larger than in EE of control hearts, thereby negatively outlining Golgi bodies (C, arrows). Pattern of actin filaments is similar to controls. D, Arrows indicate subendothelial inflammatory cells. E, In endothelium of coronary artery, 12 hours after induction of sepsis, propidium iodide labeling nearly fills entire cell and negatively outlines Golgi bodies (arrows). F-actin pattern was similar to controls and consisted of peripheral actin bundles and long stress fibers. F, Endothelium of coronary vein 12 hours after induction of sepsis showing distinct nuclei and relatively little cytoplasmic RNA labeling. Bar=10 μm.
the LPS+6h group and partially recovered but remained depressed, with a >30% decrease in +dT/dt compared with control, in the LPS+12h and LPS+36h groups. Table 2 also shows that resting tension was diminished in the LPS+36h group. Intriguingly, isometric twitches from muscles of the LPS+12h group were of markedly longer duration than twitches from the control and LPS+36h groups. By contrast, twitches of LPS+6h muscles had a tendency to be of shorter duration than those of the control group.

**Figure 2.** En face confocal microscopy of endocardial endothelium (EE) after immunostaining for NOS-2 (A through D) or COX-2 (E, F). A and B, NOS-2 staining in EE reveals fine granular cytoplasmic labeling that delineates endothelial nuclei (arrows) in LPS+12h hearts. C, 36 hours after induction of sepsis, NOS-2 labeling was much weaker than in LPS+12h hearts. D, Only very weak staining was detected in EE of control hearts. E and F, COX-2 labeling of EE was more intense 12 hours (E) than 36 hours (F) after induction of sepsis. Bars: A, 20 μm; B through F, 10 μm.

**Figure 3.** Confocal microscopy of cryostat sections of hearts 12 hours after induction of sepsis after immunostaining for NOS-2 (A through D) or COX-2 (E, F). A, Section through left ventricular myocardium shows intensely labeled endocardial endothelium (EE) (arrowheads) and weakly labeled cardiomyocytes (MYO). Endothelial cells of myocardial capillaries were not labeled above level of myocytes. B, NOS-2 labeling is distinct in endothelium of coronary arteriole (arrowheads) and in adventitial macrophage-like cells (arrows). In section, MYO were more strongly labeled than smooth muscle cells (SMC). C, Detail of longitudinally sectioned arteriole. Right, Digitalized image after thresholding pixels with gray levels between 120 and 225 reveals that arterial endothelial cells (arrowheads) were more intensely labeled than SMC and MYO. Arrows mark endothelium of intensely labeled microvessel. D, Small coronary vein. Venous endothelium (arrowheads) was weakly labeled, apparently at a level similar to MYO. E, EE cells were distinctly labeled, subendothelial cells were not labeled, and MYO were very weakly labeled for COX-2. F, Detail of arteriole showing intensely labeled endothelial cells and weakly labeled MYO. SMC were not stained. Bars: A, B, D, and E, 20 μm; C and F, 10 μm.
Our results showed that the twitch prolongation observed in the LPS+12h group coincided with the activation of cardiac endothelium seen in immunohistochemistry. Given the potential role of cardiac endothelium in modulating myocardial twitch duration, our data suggested a role of cardiac endothelium-derived mediators in the septic hearts. We therefore compared the paracrine activity of endogenous, actively released NO, PGs, and ET-1 in control and in LPS-treated hearts, in particular the LPS+12h group.

Study A (Table 3A) showed that the increase of tHR observed in the LPS+12h group was blunted by the combination of L-NMMA, indomethacin, and BQ-123 (from 463±24 to 438±22 ms, n=8, P<0.01). By contrast, the same combination had no significant effects on tHR in the control and LPS+36h groups or on AT or dT/dt in any of the control or LPS-treated hearts.

When given alone (n=5), 100 µmol/L L-NMMA exerted no effect on any measured parameter in the LPS+12h and LPS+36h groups, no effect on tHR in the control group (Table 3B), but a slight increase in AT (1.5±0.6 mN/mm², P=0.06) and in dT/dt (14±5 mN·mm⁻²·s⁻¹, P<0.05) in the control group. In separate experiments (data not shown), an excess of L-arginine (1 mmol/L) blunted the slight increase in AT and dT/dt in the control group, yet neither SOD (100 U/mL) nor a greater dose of L-NMMA (500 µmol/L) affected twitch parameters in any of the groups.

By contrast, inhibition of endogenous PGs and/or ET-1 modified cardiac relaxation in the LPS+12h group (Table 3B). Indeed, indomethacin and BQ-123, when given alone, both reduced the LPS-induced increase in tHR in LPS+12h papillary muscles (from 475±12 to 441±18 ms with indomethacin, n=5, P<0.01; from 490±29 to 468±28 ms with BQ-123, n=8, P<0.001). Experiments in which BQ-123 and indomethacin were administered to the same muscle showed that their effects were additive on tHR in the LPS+12h group whether they were given in the absence or the presence of L-NMMA (data not shown). Intriguingly, neither indomethacin nor BQ-123 had any significant effect on AT and +dT/dt in the control and LPS-treated groups (data not shown). Although the effects of endogenous PGs on tHR appeared in the LPS+12h group (compared with the other LPS-treated groups), the effects of endogenous ET-1 observed in the LPS+12h group were an amplification of the effects observed in control muscles (Table 3B).

In study B, additional papillary muscles were exposed to increasing concentrations of exogenous ET-1 (100 pmol/L to 100 nmol/L). Interestingly, ET-1 induced a larger increase in AT and a more pronounced prolongation of isometric twitches in the LPS+12h group than in the control group.

**Table 2. Baseline Contractile Performance Parameters of Rabbit Papillary Muscles**

<table>
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<tr>
<th></th>
<th>Lmax, mm</th>
<th>RT, mN/mm²</th>
<th>AT, mN/mm²</th>
<th>+dT/dt, mN·mm⁻²·s⁻¹</th>
<th>tHR, ms</th>
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<tr>
<td>Control (n=38)</td>
<td>5.00±0.18</td>
<td>9.4±0.8</td>
<td>18.1±1.6</td>
<td>111±10</td>
<td>420±14</td>
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<tr>
<td>LPS+6 h (n=8)</td>
<td>4.88±0.46</td>
<td>8.9±0.9</td>
<td>4.8±1.8*</td>
<td>34±12*</td>
<td>356±16†</td>
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<tr>
<td>LPS+12 h (n=23)</td>
<td>5.30±0.28</td>
<td>8.1±0.9</td>
<td>14.0±1.2</td>
<td>74±6*</td>
<td>482±14*</td>
</tr>
<tr>
<td>LPS+36 h (n=15)</td>
<td>4.37±0.24</td>
<td>5.4±0.6*</td>
<td>13.4±2.4</td>
<td>77±11†</td>
<td>416±14</td>
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<td>Overall P by ANOVA</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
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</table>

RT indicates resting tension at Lmax.
Multiple comparisons versus control (Dunnett’s t; *P<0.005 vs control; †P=0.08 vs control; ‡P=0.06 vs control.)
among the 3 groups: control group, 46/11002 sites B max (fmol/mg protein) of the ET A receptor were similar to trol and LPS 0.01.

12h papillary muscles. * /H11001/.

summarizing ET-1 dose-response curves on AT and tHR in con-

12h papillary muscles. B, Graphs ET-1 in control and LPS /H11001/.

A, Representative examples of effect of similar dose of

respectively (n=4); LPS+12h, 73±23 and 703±111, respectively (n=3); and LPS+36h group, 36±9 and 681±110, respectively (n=3).

Discussion

The main new findings were (1) an immunohistochemically characterized activation of endocardial and coronary arterial but not venous endothelial cells during early (12 hours) endotoxemia, (2) a concomitant prolongation of myocardial force-generating capacity that was abolished by the administration of the cyclooxygenase inhibitor indomethacin or the ET A receptor antagonist BQ-123, and (3) a sensitization of the myocardium to the twitch-prolonging and inotropic properties of ET-1. These data provide the first direct evidence of cardiac endothelial paracrine activation and intensified endothelial control of myocardial performance during sepsis.

We confirmed in this study that sepsis-related depression in myocardial performance was not explained by metabolic imbalances and was unlikely to be caused by myocardial ischemia or reperfusion injury. We further showed that resting tension at L max (ie, the preload needed to induce maximal peak isometric twitch force) was ~40% lower in muscles from LPS+36h animals than in muscles from control animals. This is in accordance with the increased ventricular compliance observed in septic animals and in septic patients.15,16

Immunohistochemical observations of hearts from endotoxemic rabbits showed clear markers of cardiac endothelial activation. NOS-2 and COX-2, which are usually not present in quiescent endothelial cells and in hearts of control rabbits, were expressed in endotoxemic hearts. This expression appeared to be transient over a period of 36 hours and was dominant in endocardial and coronary arterial endothelial cells but absent in venous endothelial, smooth muscle, and myocardial cells. In vitro and in vivo studies have shown that cardiac endothelium and its paracrine mediators (NO, PGs, ET-1, and other as yet unidentified agents) alter myocardial contractile performance mainly by influencing the time of onset of relaxation, primarily via changes in cardiac myofilament response to calcium.3,17-19 Consistently, the presented mechanical analysis revealed marked changes in the onset of

(both P<0.01) (Figure 5). In the LPS+36h group, ET-induced contractile effects were similar to those in the control group (data not shown).

Biochemical Analyses

Myocardial NOS-2 activity paralleled the transient pattern described for NOS-2 protein expression. Indeed, NOS-2 activity increased 12 hours after LPS injection compared with control (n=5 for each, 21.2±2.6 versus 7.5±0.9 cpm · μg protein⁻¹ · h⁻¹, respectively, P<0.0001) and decreased toward control levels in the LPS+36h group (n=5, 10.1±0.5 cpm · μg protein⁻¹ · h⁻¹, P<0.01 versus control).

Measurements of ET-receptor properties in LPS-treated animals showed the same results as in control animals. The dissociation constant K d (pmol) and the number of binding sites B max (fmol/mg protein) of the ET A receptor were similar among the 3 groups: control group, 46±6 and 717±64, respectively (n=4); LPS+12h, 73±23 and 703±111, respectively (n=3); and LPS+36h group, 36±9 and 681±110, respectively (n=3).

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<table>
<thead>
<tr>
<th>A. n</th>
<th>AT, mN/mm²</th>
<th>+dT/dt, mN · mm⁻² · s⁻¹</th>
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<td>n</td>
<td>BO-123</td>
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<td>-4±2</td>
<td>15</td>
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<tr>
<td>LPS+12h</td>
<td>8</td>
<td>+3±6</td>
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A. Change in contractile performance parameters (mean±SEM) from baseline by the combination of the 3 inhibitors of endothelial factors L-NMMA, BO-123, and indomethacin in papillary muscles from 3 groups of rabbits (control, LPS+12h, and LPS+36h). B. Change in tHR (ms) from baseline by inhibition of either NO, ET-1, or PG pathways.

Comparison versus baseline by paired t-test: *P<0.09; †P<0.01; ‡P<0.001.

Figure 5. A, Representative examples of effect of similar dose of ET-1 in control and LPS+12h papillary muscles. B, Graphs summarizing ET-1 dose-response curves on AT and tHR in control and LPS+12h papillary muscles. *P<0.01.
relaxation and in twitch duration of papillary muscles isolated from endotoxemic animals, which coincided with immunohistochemically observed endothelial activation. These changes were attenuated by inhibition of PGs or ET-1 and totally abolished when both factors were inhibited simultaneously. In contrast, 36 hours after endotoxin administration, when immunohistochemical signs of endothelial activation had subsided, twitch duration normalized and remained unresponsive to the same paracrine inhibitors.

The description of a paracrine effect of endothelium-derived endothelin in septic heart is novel. A similar induction of endothelin-mediated communication between the cardiac endothelium and cardiomyocytes has been described in different types of cardiac stress.26 These observations support the concept that endogenous cardiac ET-1 may be a compensatory paracrine pathway in states of myocardial dysfunction. This idea was further supported in our study by experiments with exogenous ET-1 in endotoxemic preparations that unmasked a marked sensitization of the myocardium to the inotropic and twitch-prolonging effects of ET-1 in early endotoxemia. The differences between inotropic and twitch-prolonging effects of exogenous ET-1 and the strict twitch-shortening effects of BQ-123 are in accordance with other reports showing that exogenous ET-1 does not fully reproduce the effects of endogenously released ET-1.20 Importantly, the potentiation of exogenous and endogenous ET-1 effects seen in endotoxemia appeared to be unrelated to alteration in ETα receptor density or sensitivity. Our study also confirmed sepsis-induced increases in plasma ET-1 concentration as previously described21,22 and showed that septic heart was unlikely to be the source of increased ET-1 production, because a similar pattern of ET-1 staining was found in control and septic hearts.

COX-2 was previously detected in myocardial biopsies of septic rats,23 but its cellular localization remained unknown in the heart. Our study shows that, like NOS-2, COX-2 was (1) expressed 12 hours after LPS administration, (2) localized primarily in cardiac endothelial cells and much weaker in cardiomyocytes, and (3) undetectable at 36 hours of endotoxia. In addition, COX-2 prolongs twitch duration 12 hours after LPS administration, which agrees with the previously reported twitch prolongation with arachidonic acid.18 A COX-2–induced tumor necrosis factor-α overexpression has been suggested to be involved in cardiac alteration in septic heart, via its cardiodepressant action.24 The rapid effect of indomethacin on myocardial relaxation seen in the present study, however, favors a direct and immediate action of COX-2 products in the regulation of cardiac performance in septic hearts.

Apart from the role of ET-1 and COX-related factors, our study showed a detrimental role of endothelial peroxynitrite rather than NO in myocardial performance of endotoxic hearts. Our data are consistent with previous reports that expression of NOS-2 protein, as well as calcium-independent NO production in cardiac tissue from septic animals, was increased.25 NOS inhibition, however, did not reverse the depressed cardiac function after endotoxin administration when it is established, as previously described,26–29 and myocardial function remained depressed, whereas NOS-2 protein abundance and activity had returned to control levels, in LPS +36h hearts. Consistent with other reports, the large quantities of NO synthesized by endothelial NOS-2 during sepsis could initiate a sequence of reactions leading to the formation of the strong oxidant peroxynitrite.11 This was confirmed in the present study by the similar localization of nitrotyrosine (considered a specific in vivo stable marker for the formation of peroxynitrite) and NOS-2 immunostaining in cardiac endothelium. Indeed, peroxynitrite can impair force generation indirectly by impairing mitochondrial respiration and directly by altering proteins involved in the contractile process, such as actin, and the sarcoplasmic reticulum Ca2+-ATPase.30 Accordingly, cardiac endothelial production of NOS-2–related peroxynitrite appears to be involved in long-lasting modifications of protein structure and function leading to LPS-induced cardiac dysfunction.

In summary, the present study adds novel insight into the characteristics of septic heart. In particular, it attributes a role to the cardiac endothelium in the modulation of myocardial relaxation during sepsis. Cardiac endothelial activation included the induced expression of NOS-2, colocalized with peroxynitrite and COX-2, and was restricted to coronary arterial and endocardial but not coronary venous cells, confirming cardiac endothelial cell heterogeneity.9 Functionally, cardiac endothelial cell activation resulted in prolonged twitch-force generation that was reversed by the ETA receptor antagonist BQ-123 and the COX inhibitor indomethacin. Furthermore, postreceptor signaling modifications potentiated ET-1 responses on myocardial relaxation, demonstrating that the myocardium was more receptive for cardiac endothelial control of myocardial performance in the early stage of sepsis.

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Activation of Cardiac Endothelium as a Compensatory Component in Endotoxin-Induced Cardiomyopathy: Role of Endothelin, Prostaglandins, and Nitric Oxide
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