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Lipopolysaccharide induces apoptosis in adult rat ventricular myocytes via cardiac AT\textsubscript{1} receptors

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Lipopolysaccharide (LPS) from gram-negative bacteria activates multiple cells to release cytokines, nitric oxide (NO), and other mediators with potent pathophysiological effects. Because cytokines [e.g., tumor necrosis factor-\alpha (TNF-\alpha), interleukin (IL)-1\beta, IL-6, and interferon (IFN)-\gamma] and NO depress cardiac function (21) and induce apoptosis in cardiac myocytes (24), it has been assumed that secondary mediators are responsible for the cardiotoxic effects of LPS. However, cardiac myocytes are sensitive to the direct effects of low levels of LPS, independently of mediators released from noncardiac myocytes. We found that clinically relevant levels of LPS (low ng/ml) activate cardiac myocytes within hours to depress myofilament responsiveness to calcium (41) and impair cell volume regulation (30). Cardiac myocytes may be directly sensitive to LPS due to the expression of Toll-like receptor 4 (11), the transmembrane component of the LPS receptor (3).

Apoptosis, an energy-dependent process of programmed cell death, is increased in heart failure from multiple causes (18), including sepsis (31). In sepsis, it is unknown whether cardiac apoptosis is caused by LPS itself or is a consequence of secondary effects activated by LPS. If low levels of LPS induce cardiac apoptosis, this has important implications because LPS circulates in several subacute and chronic conditions with less prominent activation of secondary mediators than in sepsis. Decompensated heart failure (32), pancreatitis, liver disease, chronic infections, smoking, and exercise (15), for example, are associated with plasma LPS levels in the picograms per milliliter to the nanograms per milliliter range.

It was hypothesized that low levels of LPS induce apoptosis in cardiac myocytes. The rationales for this hypothesis were the following points. First, cardiac apoptosis may occur in subacute or chronic conditions associated with circulating LPS. Second, blockade of secondary mediators may not prevent LPS-induced cardiac apoptosis. This is analogous to the failure for inhibitors of TNF-\alpha, IL-1\beta, platelet-activating factor, bradykinin, prostaglandins (43), and NO (14) to reduce mortality in sepsis and septic shock in several large,..
prospective, randomized, double-blind, multicenter trials (27). Finally, the mechanisms and relevance of cell activation are critically dependent on LPS level. For example, LPS induces apoptosis in association with cardiac TNF-α (6), but it requires microgram per milliliter levels of LPS to induce TNF-α release from adult cardiac myocytes (19). This is several orders of magnitude higher than LPS levels found in most clinical conditions (15, 33).

The mechanisms for LPS-induced apoptosis were examined in isolated, adult cardiac myocytes to minimize confounding secondary effects from nonmyocytes. Stringent conditions were required to limit inadvertent exposure to LPS, including depyrogenation of the digestive enzymes. Standard enzymes used for cell isolation are contaminated with 100–300 ng/ml LPS, which is sufficient to decrease the sensitivity to LPS by two to three orders of magnitude (29) and activate TNF-α synthesis (38) in cardiac myocytes. Depyrogenation of digestive enzymes allow mechanisms to be evaluated using clinically relevant levels of LPS. This study demonstrates that LPS induces apoptosis by activating AT1 receptors in cardiac myocytes. The same findings occur with LPS in vivo, with low-dose LPS (without hemodynamic effects) inducing apoptosis in left ventricular myocytes.

MATERIALS AND METHODS

Experiments were performed in accordance with the institutional guidelines and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Cardiac myocyte isolation and treatment. Adult Sprague-Dawley rats (250–400 g, either sex) were anesthetized with 40 mg/kg intraperitoneal pentobarbital sodium. The heart was excised and perfused with 150 mmol/l creatine, 5 mmol/l taurine, 1% penicillin-streptomycin and 1% phenol red, supplemented with 10 mmol/l HEPES, 3.7 mmol/l NaHCO3, 1 mg/ml glucose, 0.11 mg/ml sodium pyruvate, 2 mg/ml bovine serum albumin, 2 mmol/l L-carnitine, 5 mmol/l creatine, 5 mmol/l taurine, 1% penicillin-streptomycin, and 1% gentamycin at 37°C in 5% CO2. Myocytes were incubated for 24 h with LPS (Escherichia coli O55, LPS no. B5, lot 2039F, List Biological Laboratories; Cambell, CA) and/or angiotensin II (ANG II), preceded by 1-h exposure to metabolic inhibitors including captopril, PD-123319, Nω-monomethyl-L-arginine (L-NMMA) (all from Sigma Chemical; St. Louis, MO), losartan (a kind gift from Merck and DuPont, Rahway, NJ), TNFRII-Fc (a kind gift from Immunex; Seattle, WA), or benzyloxy carbonyl-valine-alanine-aspartate fluoromethylketone (Z-VAE-fmk, R&D Systems; Minneapolis, MN).

Assays and Western blotting. Slide-based laser scanning cytometry (Compucyte; Cambridge, MA) was performed on myocytes stained with propidium iodide (PI) and annexin V (FITC) using Apoptosis Detection kit (R&D Systems). The PI and FITC-annexin V integrals estimated the percentage of total cells that were alive (PI negative, annexin V negative) in early or late-stage apoptosis (PI dim or bright, annexin V positive) and necrosis (PI bright, annexin V negative) (20). Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assays were performed on myocytes fixed with 4% formalin phosphate-buffered saline using CardiO-TACS In Situ Apoptosis Detection kit (R&D Systems). At least 2,000 cells were scored from each group with the observer blinded to the treatment condition. Caspase-3 activity was measured with FluorAce Apopain Assay Kit (Bio-Rad Laboratories; Richmond, CA). Protein content was determined using a standard colorimetric assay (BCA, Pierce Chemical; Rockford, IL). Immunoblot assay of Bax and Bel-2 gene products were performed as described (28), with proteins quantitated by video densitometry with Alpha Innotech’s IA-200 Image Analysis software.

In vivo model. Rats were conditioned to allow measurement of blood pressure by tail cuff. Blood pressure was measured before and 15, 30, and 45 min, and 1, 2, 4, and 24 h after LPS (1 mg/kg) or saline injection into a tail vein. Rats were euthanized for heart excision. The heart was rinsed in cold saline, fixed in 3.7% formaldehyde solution for 24 h, paraffin embedded, and then sliced with 5-μm thickness cross-sections mounted on glass slides.

Photomicrographs were obtained with a digital camera (Slider Spot-2, Diagnostic Instruments; Sterling Heights, MI) mounted on an inverted microscope (Nikon Eclipse TE300; Tokyo, Japan). Images of six to eight contiguous sections of the left ventricular anterior free wall at the midventricular level were obtained to represent a transmural section. Digital images were computer processed with NIH Image to count TUNEL-positive stained cardiomyocyte nuclei and total number of nuclei in a nuclease pretreated section from the same region. The area of each section was planimetered to calculate the average transmural density of nuclei (nuclei per μm²), TUNEL-positive stained nuclei (per μm²), and rate of TUNEL-positive nuclei (per 10⁶ nuclei).

Statistical analysis. Results were compared by one- or two-way repeated measures ANOVA in protocols where myocytes from each rat were subdivided into separate dishes to test individual treatments (n = 1 for each rat heart). Results were analyzed by t-test, one- or two-way ANOVA in protocols without matched myocyte data from the same animal. Post hoc comparisons were performed with Student-Newman-Keuls methods. All results are expressed as means ± SE. Statistical significance indicates P < 0.05.

RESULTS

LPS induces apoptosis in cardiac myocytes. Adult rat ventricular myocytes were exposed for 24 h to: 1) vehicle (control), 2) LPS (100 ng/ml), or 3) a metabolic inhibition/recovery protocol (20 mmol/l 2-deoxyglucose and 1 mmol/l NaCN solution at pH 6.6 for 40 min, with recovery for 24 h), which we found to induce apoptosis (13). With the use of slide-based laser scanning cytometry as previously described (20), the percentage of myocytes in early apoptosis (positive annexin V, dimly positive PI staining) increased with LPS (12.8 ± 2.1%, means ± SE), but not with metabolic inhibition/recovery (8.9 ± 1.7%) compared with control (4.7 ± 0.5%, P < 0.05, one-way repeated measures ANOVA, n = 6). In contrast, myocytes in late apoptosis (positive annexin V, brightly positive PI staining) increased with metabolic inhibition/recovery (25.0 ± 5.9%), but not with LPS (14.5 ± 1.5%) compared with control (8.9 ± 1.3%, P < 0.05).
Apoptosis was confirmed with increased TUNEL staining from 4 to 24 h, which was greater with 10–100 ng/ml LPS (Fig. 1, \( P < 0.05 \), two-way repeated measures ANOVA on two factors, \( n = 5 \) experiments). Isolated myocyte preparations contain few (<5%) nonmyocytes (29), including cardiac fibroblasts, which are small and may overlie myocytes. This did not cause overestimation of TUNEL-stained myocytes. Cardiac fibroblasts grown to 60–80% confluence had low levels of apoptosis by PI staining (0.3–0.7% out of >10,000 cells analyzed by FACS), which was similar after exposure to 10 ng/ml LPS or vehicle for 24 h (\( n = 2 \) experiments).

Mechanisms for LPS-induced cardiac apoptosis. It was hypothesized that LPS-induced cardiac apoptosis involves ANG II because ANG II induces apoptosis in adult myocytes (17), and we found an interaction between LPS and ANG II through AT\(_1\) receptors in cardiac myocytes (42). In Fig. 2, cardiac myocytes incubated for 24 h with LPS (100 ng/ml) and/or ANG II (100 nmol/l) had similar increases in TUNEL staining without additive effects (\( P < 0.05 \) compared with control myocytes, one-way repeated measures ANOVA, \( n = 8 \) experiments). Adding losartan (1 \( \mu \)mol/l) 1 h before LPS and/or ANG II completely blocked apoptosis (\( P = \) not significant compared with control myocytes). These findings suggest that LPS induces apoptosis by activation of cardiac AT\(_1\) receptors.

These results were confirmed by measuring caspase-3 activity. In a pilot study, caspase-3 activity in myocytes increased with a peak at 16 h compared with 4, 8, and 24 h after LPS (10 ng/ml). Figure 3 shows that after 16 h, LPS (10 ng/ml) increased caspase-3 activity compared with vehicle, which was blocked by adding losartan (1 \( \mu \)mol/l) 1 h before LPS (\( P < 0.05 \), two-way repeated measures ANOVA, \( P < 0.05 \) for interaction between LPS and losartan, \( n = 10 \) experiments). ANG II (100 nmol/l) produced similar effects as LPS. In two experiments, ANG II increased caspase-3 activity 2.2- and 1.9-fold compared with vehicle.

LPS-induced apoptosis involves activation of cardiac AT\(_1\) receptors. Myocytes incubated for 24 h with LPS (10 ng/ml) had increased TUNEL staining (8.74 ± 0.39%) compared with control (5.97 ± 0.58), which was not blocked by PD-123319 (1 \( \mu \)mol/l, selective AT\(_2\) receptor inhibitor) added 1 h before LPS (8.34 ± 0.76%) (\( P < 0.05 \), one-way repeated measures ANOVA, \( n = 7 \) experiments). PD-123319 alone had no effect (5.41 ± 0.52%). In contrast, adding captopril (1 \( \mu \)mol/l) 1 h before LPS blocked the increase in TUNEL staining (5.85 ± 0.57%), whereas captopril alone had no effect (4.72 ± 0.66%) (\( n = 6 \) experiments).

Because TNF-\( \alpha \) has been reported to mediate LPS-induced apoptosis (6), myocytes were incubated for 24 h with or without LPS (10 ng/ml), soluble dimeric TNF-\( \alpha \) p75:Fc fusion protein (TNFRII:Fc, 0.5 \( \mu \)g/ml), losartan (1 \( \mu \)mol/l), or the caspase inhibitor Z-VAD-fmk (100 \( \mu \)mol/l) (inhibitors added 1 h before LPS). Figure 4 shows LPS-induced apoptosis was blocked by losartan and Z-VAD-fmk (\( P < 0.05 \), one-way repeated measures ANOVA, \( n = 8 \) experiments), but not by...
TNFRII:Fc. Thus low-dose LPS activated AT₁ receptors to induce apoptosis, without involving TNF-α.

LPS-induced apoptosis was not NO mediated. The increase in TUNEL staining at 24 h with 10 ng/ml LPS (8.45 ± 2.55%) compared with control (5.40 ± 1.47%) was not blocked by adding the NO synthase inhibitor l-NMMA (1 mmol/l) 1 h before LPS (8.30 ± 1.83%), whereas l-NMMA alone had no effect (4.48 ± 0.86%) (P < 0.05, two-way repeated measures ANOVA, n = 4 experiments).

Both LPS (10 ng/ml) and ANG II (100 nmol/l) decreased the ratio of the antiapoptotic protein Bcl-2 relative to the apoptotic protein Bax (Fig. 5). This was primarily related to a decrease in Bcl-2, with little or no change in Bax. At 12 h (time of peak change in a pilot study), Bcl-2 decreased 28 ± 7% with LPS and 40 ± 6% with ANG II compared with vehicle (control). In both cases, changes in Bcl-2 were greater than changes in Bax (P < 0.05, one-way ANOVA, n = 6 experiments), with no difference between LPS and ANG II responses.

**LPS induces cardiac apoptosis in vivo.** The in vivo effects of LPS were evaluated by injecting either LPS (1 mg/kg) or saline into a tail vein (n = 6 rats each) and then examining the heart after 24 h for apoptosis in left ventricular nuclei. Neither LPS nor saline caused distress or affected systolic blood pressure after 15, 30, or 45 min, and 1, 2, 3, 4, 5, or 24 h (measured in 4 rats with each treatment). After 24 h, the rate of TUNEL-positive stained nuclei in the left ventricle was 1.9 ± 0.3-fold higher in LPS-treated than saline-treated rats (610 ± 87 vs. 362 ± 70 positive nuclei/10⁶ nuclei, P = 0.05, t-test).

The time course for apoptosis was studied in rats at 12 h, 1, 2, 3, 7, and 14 days after a single injection of LPS (1 mg/kg) or saline (n = 3 rats each with LPS or saline at each time period). Figure 6 shows that a single injection of LPS increased TUNEL-stained nuclei for 12 h to 3 days, but not after 1–2 wk compared with saline controls (Fig. 6, P < 0.05, univariate ANOVA with test of linear trends).

To determine whether LPS induced cardiac apoptosis in vivo by the same AT₁ receptor-mediated mechanism as LPS in vitro, four groups of rats (n = 6 per group) were injected by tail vein with: 1) saline (control), 2) LPS (1 mg/kg), 3) saline after 3 days pretreatment with losartan (23 ± 2 mg·kg⁻¹·day⁻¹ added to drinking water) (losartan group), or 4) LPS (1 mg/kg) after 3 days pretreatment with losartan (LPS + losartan group). Three days of losartan pretreatment decreased systolic blood pressure slightly from 119 ± 1 to 117 ± 1 mmHg (P < 0.05, paired t-test). Neither LPS nor saline injections caused distress or altered systolic blood pressure.

**Fig. 4.** TUNEL staining (means ± SE, n = 8) in cardiac myocytes incubated with vehicle (control), LPS (10 ng/ml), tumor necrosis factor-α (TNF-α) p75:Fc fusion protein (TNFRII:Fc, 0.5 μg/ml), losartan (1 μmol/l), or benzyloxycarbonyl-valine-alanine-aspartate fluoromethylketone (Z-VAD-fmk, 100 μmol/l) for 24 h. LPS-increased TUNEL staining that was not blocked by TNFRII:Fc but was blocked by losartan or Z-VAD-fmk.

**Fig. 5.** Cardiac myocytes incubated for 12 h with vehicle (control), LPS (10 ng/ml), and ANG II (100 nmol/l). Representative raw gel data are shown on top with group data (means ± SE, n = 6) below. Both LPS and ANG II induced greater changes (compared with control) in the antiapoptotic protein Bcl-2 than the apoptotic protein Bax (P < 0.05), with no difference between LPS and ANG II effects for either Bcl-2 or Bax.

**Fig. 6.** A single intravenous injection of LPS (1 mg/kg) in vivo increased TUNEL-stained left ventricular nuclei for 3 days (but not 1–2 wk) compared with saline-injected control rats (Fig. 6, P < 0.05, univariate ANOVA with test of linear trends).
blood pressure between 15 min and 24 h in any group, as shown in Table 1 (P = 0.001, one-way repeated measures ANOVA). Figure 7 shows that LPS increased TUNEL staining in left ventricular nuclei after 24 h compared with control, which was blocked in rats pretreated with losartan (P < 0.001, two-way ANOVA, P < 0.01 for interaction between LPS and losartan). Thus, similar to in vitro results, LPS in vivo induced an approximately twofold increase in apoptosis in cardiac nuclei after 24 h, which was blocked by inhibiting AT_1 receptors.

**DISCUSSION**

This study demonstrated that low levels of LPS induce apoptosis in cardiac myocytes in vitro and in vivo by activation of cardiac AT_1 receptors. In cardiac myocytes, LPS decreased the ratio of anti-apoptotic Bcl-2 to proapoptotic Bax proteins at 12 h; increased caspase-3 activity at 16 h; and increased apoptosis at 24 h with twofold increases in annexin V, propidium iodide, and TUNEL staining. ANG II produced similar changes as LPS without additive effects. LPS-induced apoptosis was completely blocked by inhibiting AT_1 receptors (losartan) or angiotensin-converting enzyme (captopril), but not by inhibiting AT_2 receptors (PD-123319).

LPS in vivo also induced an approximately twofold increase in apoptosis at 24 h, which was abolished by losartan. A single dose of LPS in vivo that caused no distress and did not affect blood pressure was sufficient to increase cardiac apoptosis for 1–3 days. LPS induced apoptosis in left ventricular myocytes with rates of ~600–700 nuclei per 10^6 nuclei. This is similar to apoptotic rates (<1%) found in several human and animal models of acute and chronic heart failure (18). The significance of these rates depends on the duration of apoptosis. If apoptosis is completed within 24 h, for example, the percentage of myocytes undergoing apoptosis over 3 days would be threefold higher than the rate of apoptosis measured at a single time point. In conditions associated with recurrent or chronic exposure to subclinical levels of LPS (e.g., teeth cleaning, smoking, periodontal disease, and chronic infections), recurrent episodes of apoptosis may lead to cumulative damage.

Table 1. Systolic blood pressures with and without LPS and losartan over a 24-h period

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>LPS</th>
<th>Losartan</th>
<th>LPS + Losartan</th>
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<tr>
<td>0 min</td>
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<td>115 ± 2</td>
<td>116 ± 1</td>
<td>115 ± 1</td>
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<tr>
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<td>113 ± 2</td>
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<td>114 ± 3</td>
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<tr>
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<td>120 ± 2</td>
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<tr>
<td>24 h</td>
<td>118 ± 2</td>
<td>118 ± 2</td>
<td>116 ± 1</td>
<td>117 ± 3</td>
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Systolic blood pressure (mmHg, means ± SE) measured by tail cuff did not change after injecting saline (control) or lipopolysaccharide (LPS) (1 mg/kg) into the tail vein in four groups of rats (n = 6/group). In two of the groups, losartan (23 mg·kg⁻¹·day⁻¹) was added to the drinking water for 3 days before LPS or saline injections.

LPS may activate cardiac myocytes to release TNF-α or NO to mediate apoptosis in an autocrine manner. However, neither TNF-α nor NO contributed significantly to the apoptosis induced by low levels of LPS. High micrograms per milliliter of LPS induce TNF-α production in cardiac myocytes in vivo and in vitro (12, 19). This may cause apoptosis that is blocked by TNFRII:Fc (6). TNF-α may have a bifunctional role and also protect against cardiac apoptosis (26). In the current study, TNF-α played a role in mediating apoptosis induced by low levels of LPS. LPS-induced TNF-α did not play a protective role either, because TNFRII:Fc did not exacerbate apoptosis.

The minimal role of cardiac TNF-α is not surprising because overexpression of TNF-α in a transgenic model is associated with only rare myocyte apoptosis (25), and TNF-α alone does not induce apoptosis in neonatal cardiac myocytes in vitro (16). Furthermore, it requires three orders of magnitude higher LPS doses to induce TNF-α release from adult compared with neonatal myocytes (10 μg/ml vs. 10 ng/ml LPS) (39), which are well beyond LPS levels in most clinical conditions. The stringent measures used in this study to minimize LPS exposure, including enzyme deproteination, may modify the role of LPS-induced TNF-α. Otherwise, exposure to LPS contaminants in collagenase can cause baseline activation of TNF-α in isolated cardiac myocytes during cell isolation (39). TNF-α did not mediate apoptosis induced by low levels of LPS, but this does not exclude contributions by TNF-α under other circumstances. For example, high levels of LPS may up-regulate cardiac TNF-α and contribute to apoptosis by additional mechanisms.

Low levels of LPS activate NO-mediated pathways in cardiac myocytes (30, 41), and cytokine-induced NO can induce apoptosis in neonatal (16) and adult cardiac myocytes (1). NO can play a bifunctional role with either proapoptotic or antiapoptotic effects (22). In this study, the NO synthase inhibitor L-NMMA neither
attenuated nor exacerbated LPS-induced apoptosis, indicating that cardiac NO was not a major mediator.

It was hypothesized that LPS-induced apoptosis is mediated through the cardiac renin-angiotensin system. ANG II activates AT1 receptors to induce apoptosis in neonatal (5) and adult (17, 35) cardiac myocytes. This is blocked by losartan, but not by PD-123319. ANG II induces caspase-3 activation in adult cardiac myocytes (35). In spontaneously hypertensive rats, apoptosis is blocked by captopril or losartan, with all examined in the current study. However, LPS-induced apoptosis increases angiotensin-converting enzyme or AT1 receptors on LPS-induced apoptosis. The effects of inhibitors of angiotensin-converting enzyme (7). This is associated with overexpression of Bax-α protein and is inhibited by losartan (10). In diabetic cardiomyopathy (streptozotocin model), cardiac myocyte apoptosis increases in association with upregulation of angiotensinogen, renin, AT1 receptors, and ANG II production, which are attenuated with losartan (9).

Cardiac myocytes contain key components of tissue renin-angiotensin, including angiotensinogen, renin, angiotensin-converting enzyme, and AT1 receptors (8). Stretch of cardiac myocytes causes release of ANG II in a bimodal pattern (initial peak at 1 h, slower increase over 20 h) to induce apoptosis by a p53-mediated mechanism that is blocked by losartan (28). The tumor suppressor protein p53 triggers apoptosis by increasing cardiac myocyte expression of angiotensinogen, AT1 receptors, and increasing Bax relative to Bcl-2 (34). ANG II increases the ratio of Bax to Bcl-2 protein, which is attenuated, but not eliminated, by losartan (35). This suggests that changes in Bax and Bcl-2 contribute to, but are not solely responsible for, ANG II-induced apoptosis. The effects of inhibitors of angiotensin-converting enzyme or AT1 receptors on LPS-induced changes in Bcl-2 and Bax protein were not examined in the current study. However, LPS-induced apoptosis was blocked by captopril or losartan, with all LPS effects mimicked by ANG II.

The novel finding in this study is that LPS activates tissue renin-angiotensin to induce apoptosis in cardiac myocytes. There are scant data implicating LPS activation of the cardiac renin-angiotensin system. LPS in vivo increases angiotensinogen mRNA after 9–17 h in several organs, including the heart (23). LPS downregulates AT2 receptors in cardiac fibroblasts (36) but increases AT1 receptors in vascular smooth muscle (4). In contrast to these sparse reports for LPS, there is considerable evidence for activation of the cardiac renin-angiotensin system by humoral (e.g., glucocorticoids, estrogen, and thyroid hormone) and mechanical (e.g., myocyte stretch or altered wall stress) factors (8).

The current and prior studies demonstrate that cardiac myocytes are highly sensitive to low nanograms per milliliter of LPS, which depress myofilament sensitivity to calcium (41), impair cell volume regulation (30), and induce apoptosis. LPS activates cells by a complex interplay between soluble and cell surface recognition proteins for LPS (e.g., LPS binding protein, soluble CD14, and membrane-bound CD14) (37) and Toll-like receptor 4. Toll-like receptor 4 plays a key role in transmembrane signaling to LPS (3) and is expressed abundantly on cardiac myocytes (11). These receptors make cells sensitive to low levels of LPS by activating unique signaling pathways. Higher levels of LPS may overwhelm this system to activate cardiac myocytes by nonspecific mechanisms that lack clinical relevance for most conditions.

The direct cardiac effects of LPS are clinically relevant because picograms per milliliter to low nanograms per milliliter of plasma LPS levels occur acutely in bacteremia, sepsis, septic shock, pancreatitis, and acute respiratory distress syndrome (15, 33), and subacutely in decompensated heart failure (32), cirrhosis, chronic infections (e.g., lung, urinary tract or periodontal disease), and smoking (40). In this study, low doses of LPS in vivo caused no distress or change in blood pressure but were sufficient to induce cardiac apoptosis for days. Apoptosis may contribute to the cumulative loss of cardiac myocytes because myocytes are postmitotic cells [notwithstanding recent evidence to the contrary (2)]. The importance of a twofold increase in cardiac apoptosis may be magnified in several conditions associated with recurrent or chronic circulating LPS.

In summary, clinically relevant levels of LPS directly induce cardiac myocytes to undergo apoptosis by activation of the cardiac renin-angiotensin system. Selective blockade of AT1 receptors effectively blocks LPS-induced cardiac apoptosis in vitro and in vivo. In conditions with sustained or recurrent endotoxemia, LPS may accelerate the loss of cardiac myocytes by apoptosis.

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REFERENCES


