Increased levels of myocardial I<sub>KB</sub>-α protein promote tolerance to endotoxin

Shames, Brian D., Daniel R. Meldrum, Craig H. Selzman, Edward J. Pulido, Brian S. Cain, Anirban Banerjee, Alden H. Harken, and Xianzhong Meng. Increased levels of myocardial I<sub>KB</sub>-α protein promote tolerance to endotoxin. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1084–H1091, 1998.—Endotoxin [lipopolysaccharide (LPS)] causes tumor necrosis factor-α (TNF-α)-mediated myocardial contractile depression. Tolerance to the cardiac toxicity of LPS can be induced by a prior exposure to LPS or by pretreatment with glucocorticoids. The mechanisms by which the myocardium acquires tolerance to LPS remain unknown. LPS causes phosphorylation and degradation of inhibitory κB (I<sub>KB</sub>-α), releasing nuclear factor-κB (NF-κB) to activate TNF-α gene transcription. We hypothesized that LPS induces supranormal synthesis of myocardial I<sub>KB</sub>-α protein and thus renders the myocardium tolerant to subsequent LPS. Rats were challenged with LPS after pretreatment with LPS, dexamethasone, or saline. In saline-pretreated rats, LPS caused a rapid decrease in myocardial I<sub>KB</sub>-α protein levels, activation of NF-κB, and increased TNF-α production. These events were followed by myocardial contractile depression. After the initial decrease in myocardial I<sub>KB</sub>-α, I<sub>KB</sub>-α protein levels rebounded to a level greater than control levels by 24 h. Dexamethasone pretreatment similarly increased myocardial I<sub>KB</sub>-α protein levels. In rats pretreated with either LPS or dexamethasone, myocardial I<sub>KB</sub>-α protein levels remained similar to control levels after LPS challenge. The preserved level of myocardial I<sub>KB</sub>-α protein was associated with diminished NF-κB activation, attenuated myocardial TNF-α production, and improved cardiac contractility. We conclude that LPS and dexamethasone upregulate myocardial I<sub>KB</sub>-α protein expression and that an increased level of myocardial I<sub>KB</sub>-α protein may promote cardiac tolerance to LPS by inhibition of NF-κB intranuclear translocation and myocardial TNF-α production.

nuclear factor-κB; tumor necrosis factor-α; cardiac contractility; glucocorticoids; rat

ENDOTOXIN [lipopolysaccharide (LPS)] causes transient myocardial contractile depression (1, 13, 28, 30), which is mediated, at least in part, by tumor necrosis factor-α (TNF-α) (11, 18). We and others have observed (6, 23, 25, 26) that LPS induces cardiac functional tolerance to a subsequent ischemic insult. Our recent work (28) demonstrated that a prior exposure to LPS also induces tolerance to subsequent endotoxemic myocardial depression. However, the mechanisms of induced myocardial tolerance to LPS remain unknown.

LPS stimulates monocytes and macrophages by binding to CD14 receptors (43). Signals distal to the CD14 receptors activate nuclear factor-κB (NF-κB) (29). NF-κB is a family of proteins that share a common Rel domain that regulates nuclear translocation and gene transcription of multiple proinflammatory cytokines (36), including TNF-α (8, 35). Myocardial NF-κB is therefore a logical target for designing strategies to promote cardiac tolerance to LPS.

Cytosolic association with inhibitory κB (I<sub>KB</sub>) prevents NF-κB intranuclear translocation and DNA binding. The I<sub>KB</sub> family of proteins is characterized by an ankyrin repeat domain that allows for binding to the nuclear localization sequence of NF-κB (4). The I<sub>KB</sub>-α gene contains an NF-κB binding site in its promoter, which permits regulation of I<sub>KB</sub>-α expression by NF-κB (14, 22). Multiple stimuli, including LPS, activate NF-κB by initiating phosphorylation of I<sub>KB</sub>. Phosphorylation of I<sub>KB</sub>-α on two specific serine residues identifies it for ubiquitination and subsequent degradation by the 26S proteasome, permitting intranuclear translocation of NF-κB with resultant cytokine gene transcription (3, 36). LPS stimulation, both in vivo and in vitro, causes a rapid degradation with subsequent resynthesis of I<sub>KB</sub>-α in various cell types. This resynthesis is an inducible autoregulatory pathway that functions to turn off NF-κB-activated gene transcription (37). The temporal profile of myocardial I<sub>KB</sub>-α after LPS exposure has not been delineated. Examination of the temporal profile of I<sub>KB</sub>-α in the myocardium may provide insight into the mechanisms by which the myocardium adapts to stress.

We have recently observed that glucocorticoids inhibit LPS-induced myocardial TNF-α production (26) and prevent endotoxemic myocardial depression (27). Glucocorticoids inhibit NF-κB-mediated gene transcription in cultured monocytic cells and lymphocytes by inducing I<sub>KB</sub>-α (2, 33). However, it is unknown whether glucocorticoids upregulate myocardial I<sub>KB</sub>-α in vivo. It has been postulated (5, 17, 38, 44) that LPS tolerance is mediated through inhibition of NF-κB-dependent gene transcription. We hypothesized that myocardial I<sub>KB</sub>-α protein expression is enhanced in LPS-tolerant hearts and that upregulation of myocardial I<sub>KB</sub>-α attenuates the NF-κB-mediated myocardial response to subsequent LPS.

The purposes of this study were 1) to delineate the temporal profile of myocardial I<sub>KB</sub>-α protein expression after LPS challenge in both tolerant and naive rats, 2) to determine whether tolerance-inducing stimuli enhance myocardial I<sub>KB</sub>-α protein expression, 3) to examine the influence of LPS pretreatment on LPS-induced myocardial NF-κB DNA binding, intranuclear translocation, and TNF-α production, and 4) to relate myocardial I<sub>KB</sub>-α protein expression, NF-κB activity, and TNF-α content to cardiac contractile function after LPS challenge.
**MATERIALS AND METHODS**

Animals. Male Sprague-Dawley rats, body weight 300–325 g, were quarantined and maintained on a standard pellet diet for 2 wk before initiation of the experiments. All animal experiments were approved by the Animal Care and Research Committee of the University of Colorado Health Sciences Center. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892].

Chemicals and reagents. Dexamethasone was purchased from Elkins-Sinn (Cherry Hill, NJ). The TNF-α assay kit was obtained from Genzyme (Cambridge, MA). All antibodies for immunoblotting and immunohistochemistry were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The IκB-α antibody is a rabbit polyclonal IgG against the carboxy terminus of human IκB-α and cross-reacts with rat IκB-α. The NF-κB antibodies are both goat polyclonal IgG raised against the carboxy terminus of the human p65 or p50 subunit of NF-κB, and both cross-react with the rat NF-κB subunits. The enhanced chemiluminescence (ECL) kit was obtained from Amersham (Arlington Heights, IL). LPS (from Salmonella typhimurium) and all other chemicals were purchased from Sigma Chemical (St. Louis, MO).

Experimental protocols. Rats were pretreated with LPS (dissolved in bacteriostatic normal saline, 0.5 mg/kg ip, 24 h), dexamethasone (8 mg/kg iv, 30 min) or saline (0.4 ml ip, 24 h, or 0.4 ml iv, 1 h). We have previously demonstrated that this dose of LPS induces cardiac tolerance to subsequent LPS challenge (28). After pretreatment, rats were challenged with LPS (0.5 mg/kg ip). Hearts were rapidly excised after anesthesia with pentobarbital sodium (Nembutal, 60 mg/kg ip) and anticoagulation with heparin sodium (500 units ip) and were subjected to immunoblotting for IκB-α (1–24 h after LPS challenge, 3 hearts in each group), electrophoretic mobility shift assay (EMSA) for NF-κB (1–4 h after LPS challenge, 3 hearts in each group), immunofluorescent localization of NF-κB (1–4 h after LPS challenge, 2 hearts in each group), TNF-α assay (1–4 h after LPS challenge, 6 hearts in each group), or isolated heart perfusion (6 h after LPS challenge, 6 hearts in each group).

Immunoblotting. After excision, each heart was flushed by retrograde perfusion through the aortic root with 10 ml of cold (4°C) PBS, and major vessels and atria were removed. Ventricular tissue was frozen with liquid N2 and stored at −70°C. Myocardium was homogenized with a Tissumizer (Tekmar, Cincinnati, OH) in 5 vol of homogenization buffer containing (in mM) 25 Tris·HCl, 2 EGTA, 1 benzamidine, and 1 phenylmethylsulfonyl fluoride (PMSF), pH 7.4. After centrifugation at 3,000 g at 4°C for 20 min, the supernatant was collected. Protein concentration was determined using the Lowry assay (21). Samples (20 µg of crude protein) were mixed with an equal volume of sample buffer (100 mM Tris·HCl, 2% SDS, 0.02% bromophenol blue, and 10% glycerol) and boiled. Electrophoresis was performed on 4–20% linear gradient SDS polyacrylamide gels. Proteins were then electrophoretically transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA). Membranes were blocked for 1 h at room temperature with antibody buffer (PBS containing 0.1% Tween 20 and 5% nonfat dried milk) and then incubated with primary antibody (rabbit polyclonal anti-IκB-α, 1:500 dilution with antibody buffer) for 1 h at room temperature. Membranes were washed three times in PBS containing 0.1% Tween 20 and then incubated with peroxidase-labeled goat anti-rabbit IgG (1:10,000 dilution with antibody buffer) for 1 h at room temperature. Membranes were then washed three times, and antigen-antibody complexes were revealed by ECL.

Quantification of the immunoblot was performed by computer-assisted densitometry (NIH Application 1.599b4). Density values are expressed as a percentage of the saline control level of each experiment. All densities reported are means ± SE of three separate experiments.

EMSA. After excision, each heart was flushed and major vessels and atria were removed. Nuclear extracts were then prepared using a modified technique of Schreiber et al. (34). Hearts were homogenized in 5 vol of homogenate buffer containing 10 mM HEPES (pH 7.9), 10 mM KCl, 0.5 M sucrose, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, 0.1% Tween 20 and then incubated with primary antibody (rabbit polyclonal anti-IκB-α) for 1 h at room temperature with antibody buffer (PBS containing 0.1% Tween 20 and 5% nonfat dried milk) and then incubated with peroxidase-labeled goat anti-rabbit IgG (1:10,000 dilution with antibody buffer) for 1 h at room temperature. Membranes were then washed three times, and antigen-antibody complexes were revealed by ECL.

FIG. 1. Immunoblotting of myocardial inhibitory κB-α (IκB-α) protein. Rats were challenged with lipopolysaccharide (LPS; 0.5 mg/kg ip) after pretreatment with normal saline (NS; A), LPS (0.5 mg/kg ip, 24 h; B), or dexamethasone (Dex; 8 mg/kg iv, 30 min; C). Hearts were excised at sequential time points after LPS challenge, and myocardial IκB-α protein was probed by immunoblotting. Representative immunoblots of 3 separate experiments are shown. A: myocardial IκB-α protein was decreased at 2 and 4 h after LPS challenge in NS-pretreated rats. Myocardial IκB-α protein was normalized at 4 h and was increased above control level (NS pretreated/NS challenge) at 24 h. B: in rats pretreated 24 h earlier with LPS, myocardial IκB-α levels at 1–4 h after LPS challenge were similar to those of LPS-pretreated/NS-challenged control. C: another group of rats was pretreated with Dex and then challenged with LPS. Dex pretreatment alone induced myocardial IκB-α, and myocardial IκB-α remained similar to Dex-pretreated/NS-challenged control level at 1–2 h after LPS challenge in Dex-pretreated animals. All experiments were performed at the same time, allowing comparisons among individual blots.
(DTT), 1 mM benzamidine, and 1 mM PMSF. Homogenates were then centrifuged at 750 g for 10 min at 4°C to isolate crude nuclei (20). The crude nuclear pellet was then resuspended in 100 ml of ice-cold nuclear extraction buffer [20 mM HEPES (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM benzamidine, and 1 mM PMSF], and the tube was placed on ice for 30 min with brief, gentle vortexing every 5 min. The nuclear extract was then centrifuged at 12,000 g for 5 min at 4°C. The supernatant was collected and the protein quantified using the Lowry assay.

NF-κB consensus oligonucleotide (5'-AGTTGAGGG ACTTTCCCCAGGC-3', binding site underlined) was 5' end labeled with [γ-32P]ATP using T4 polynucleotide kinase. Unincorporated nucleotide was removed using a NucTrap Probe purification column (Stratagene, La Jolla, CA). Ten micrograms of nuclear protein were incubated with labeled oligonucleotide (100,000–200,000 counts/min) in binding buffer [10 mM Tris·HCl (pH 7.5), 50 mM NaCl, 0.5 mM EDTA, 1 mM MgCl2, 0.5 µg poly(dL-dC)-poly(dL-dC), 1% Nonidet P-40, and 4% glycerol] for 25 min at room temperature in a final volume of 25 µl. Subsequently, the free oligonucleotide and oligonucleotide-bound proteins were separated by electrophoresis on a native 4% polyacrylamide gel. The gel was then dried and exposed to an X-ray film with intensifying screens overnight at −70°C.

For supershift studies, antibodies (1 µg) to either p50 or p65 were added before the addition of labeled oligonucleotide. Binding of the antibody to NF-κB was indicated by a supershift in the EMSA. To further demonstrate specificity, excess unlabeled oligonucleotide was used as a specific competitor.

Immunohistochemistry. After excision, hearts were flushed as mentioned in Immunoblotting, and the ventricular tissue was embedded in tissue freezing medium. Tissue was then rapidly frozen in dry ice-cooled 2-methylbutane and stored at −70°C. Transverse 5-mm cryosections were prepared with a cryostat (IEC Minotome plus, Needham Heights, MA) and collected on poly-L-lysine-coated slides. All sections were fixed for 10 min in a 70% acetone-30% methanol mixture at −20°C. Sections were then blocked with 10% normal goat serum and incubated with primary antibody (rabbit polyclonal anti-NF-κB p65, 1:40 dilution with PBS containing 1% bovine serum albumin) for 1 h. After three washes with PBS, sections were incubated for 45 min with Cy3-labeled goat anti-rabbit IgG (1:250 dilution with PBS containing 1% bovine serum albumin). After being washed with PBS, sections were counterstained with fluorescein-labeled wheat germ agglutinin (5 µg/ml, for cell surface staining) and bisbenzimide (2.5 µg/ml, for nuclear staining). Sections were then mounted with aqueous anti-quenching medium. To assess the specificity of the immunostaining, adjacent sections were incubated with nonimmune rabbit IgG (5 µg/ml in PBS containing 1% bovine serum albumin) in replacement of the primary antibody and then processed identically. Microscopic observation and photography were performed with a Leica DMRXA confocal microscope (Germany).

TNF-α assay. TNF-α was measured in myocardial homogenates using an ELISA system containing a hamster antimouse TNF-α antibody (which cross-reacts with rat TNF-α). Recombinant murine TNF-α was used as a standard. Absorbances of standards and samples were determined spectrophotometrically at 450 nm using a microplate reader (Bio-Rad, Hercules, CA). Results were recorded as optical densities and plotted against the linear portion of the standard curve. Protein levels in myocardial homogenates were measured using the Lowry assay. Results are presented in picograms of TNF-α per milligram of myocardial protein.

Isolated heart perfusion and assessment of cardiac function. Cardiac function was determined by an isovolumetric Langendorff technique as described previously (28) and expressed as left ventricular developed pressure (LVEDP). Hearts were excised into cold (4°C) modified Krebs-Henseleit solution containing (in mM) 5.5 glucose, 119 NaCl, 1.2 CaCl2, 4.7 KCl, 25 NaHCO3, 1.18 KH2PO4, and 1.17 MgSO4. The aorta was cannulated, and the heart was perfused with 30 s after isolation. Hearts were perfused in the isovolumetric mode (70 mmHg) with the modified Krebs-Henseleit solution, which was saturated with 92.5% O2-7.5% CO2 to achieve a Po2 of 440–460 mmHg, a Pco2 of 39–41 mmHg, and a pH of

![Fig. 2. Densitometric quantification of myocardial IκB-α level.](http://ajpheart.physiology.org/content/103/2/32.247)

**A**: densitometry analysis reveals that LPS causes a decrease in IκB-α protein levels in NS-pretreated animals and then an upregulation above control levels by 24 h. B: when LPS-pretreated animals are subsequently challenged with LPS, IκB-α levels remain similar to control levels. C: Dex pretreatment similarly increases IκB-α levels above control levels. When Dex-pretreated animals are challenged with LPS, IκB-α levels remain similar to control levels. *P < 0.05 vs. NS-pretreated/NS-challenged control.
7.39–7.41 (determined by ABL-4 blood gas analyzer, Copenhagen, Denmark). A latex balloon was inserted through the left atrium into the LV, and the balloon was filled with water to achieve an LV end-diastolic pressure (LVEDP) of 5–10 mmHg. Pacing wires were fixed to the right atrium, and the heart was paced at 6.0 Hz (350 beats/min). Hearts were perfused for 15 min, and LVDP, LVEDP, and heart rate were continuously recorded with a computerized pressure amplifier/digitizer (MacLab 8, ADInstruments, Milford, MA).

Statistical analysis. Data are presented as means ± SE. ANOVA was performed to analyze differences among experimental groups. Statistical significance was accepted within 95% confidence limits.

RESULTS

Myocardial IκB. To delineate the temporal profile of myocardial IκB-α after LPS challenge, saline-pretreated rats were challenged with LPS and myocardial IκB-α protein level was determined using immunoblotting. LPS challenge caused a decrease in myocardial IκB-α within 1 h. Myocardial IκB-α remained lower than control level at 2 h and returned to control level by 4 h. However, myocardial IκB-α rebounded above control level at 24 h after LPS treatment (Figs. 1A and 2A).

To examine the influence of LPS pretreatment on myocardial IκB-α after subsequent LPS challenge, rats pretreated with LPS 24 h earlier were challenged with LPS and myocardial IκB-α was determined at 1, 2, and 4 h after challenge. As presented in Figs. 1B and 2B, myocardial IκB-α remained similar to control levels at all of these time points. To examine whether glucocorticoids also induce myocardial IκB-α and preserve myocardial IκB-α, rats were treated with dexamethasone and myocardial IκB-α was determined in a group of rats at 1 h after dexamethasone treatment and in other groups of rats at 1 and 2 h after subsequent LPS challenge. These results are presented in Figs. 1C and 2C. Dexamethasone induced an increase in myocardial IκB-α. When dexamethasone-pretreated rats were challenged with LPS, IκB-α remained similar to control levels.

EMSA for NF-κB. EMSA was performed on myocardial nuclear extracts after LPS challenge. NF-κB was activated at 1–2 h after LPS challenge in saline-pretreated rats. NF-κB DNA binding activity declined 4 h after LPS challenge, although not back to control levels. In LPS-pretreated rats, NF-κB activation after subsequent LPS challenge was attenuated (Fig. 3A). Supershift experiments revealed that both p50 and p65 subunits of NF-κB were involved in DNA binding (Fig. 3B). Binding specificity was confirmed by including a 100-fold excess of unlabeled consensus oligonucleotide in the DNA binding reaction. The addition of unlabeled oligonucleotide greatly diminished the intensity of the shifted band (Fig. 3B).

Immunofluorescent analysis of myocardial NF-κB. To determine which cell types were involved in myocardial NF-κB activation, we examined the cellular and subcellular distribution of NF-κB in the myocardium by immunofluorescent localization. NF-κB immunoreactivity was not detected on sections incubated with nonimmune rabbit IgG (data not shown). Immunostaining with rabbit polyclonal anti-NF-κB p65 detected NF-κB immunoreactivity in both interstitial cells and myocytes. The results of NF-κB localization are presented in Fig. 4. Control hearts from untreated rats had minimal nuclear NF-κB. In saline-pretreated animals, LPS challenge resulted in the appearance of intranuclear NF-κB at 1 (data not shown) and 2 h after LPS challenge (Fig. 4). NF-κB remained in the nucleus at 4 h (data not shown). NF-κB translocation primarily involved cardiac interstitial cells, although intranuclear...
NF-κB was also observed in myocytes. This suggests that cardiac interstitial cells are the main source of myocardial TNF-α. In LPS-pretreated rats, subsequent LPS challenge resulted in a markedly reduced intranuclear translocation of NF-κB at 2 h (Fig. 4). This observation is consistent with the gel shift data demonstrating activation of NF-κB in naive rats challenged with LPS and attenuation in LPS-pretreated rats.

Myocardial TNF-α. To examine the influence of induced IκB-α on myocardial TNF-α production, TNF-α was measured in myocardial homogenate after LPS challenge in animals pretreated with saline, LPS, or dexamethasone. In saline-challenged rats, myocardial TNF-α was 5.3 ± 0.5 pg/mg. LPS challenge increased myocardial TNF-α at 1 and 2 h in saline-pretreated animals (26.1 ± 3.8 and 33.1 ± 6.5 pg/mg, respectively; both P < 0.05 vs. saline-challenged rats). TNF-α levels peaked at 2 h and declined to control level by 4 h (Fig. 5A). Pretreatment with either LPS or dexamethasone inhibited the peak myocardial TNF-α production (Fig. 5B). Myocardial TNF-α 2 h after LPS challenge was 13.9 ± 3.8 pg/mg in LPS-pretreated rats (P < 0.05 vs. saline-pretreated rats) and 12.8 ± 3.5 pg/mg in dexamethasone-pretreated rats (P < 0.05 vs. saline-pretreated rats).

Cardiac contractile function. To examine the influence of induced IκB-α on contractile function, rats were challenged with LPS after pretreatment with LPS, dexamethasone, or saline, and contractility was assessed. We have previously demonstrated that maximal contractile depression occurs at 6 h after LPS challenge (28). Therefore, hearts were excised, and contractility was assessed 6 h after LPS challenge. As shown in Fig. 6, LVDP was 99.1 ± 2.3 mmHg in saline-challenged rats. LPS challenge resulted in significant depression of myocardial contractility in rats pretreated with saline (LVDP = 57.2 ± 3.4 mmHg, P < 0.001 vs. saline-challenged animals). In contrast, pretreatment with either LPS or dexamethasone preserved cardiac contractility after LPS challenge. LVDP was 101.7 ± 3.4 mmHg in LPS-pretreated rats after LPS challenge (P < 0.001 vs. saline-pretreated rats) and 93.8 ± 2.4 mmHg in dexamethasone-pretreated rats (P < 0.001 vs. saline-pretreated rats).
DISCUSSION

The myocardium overexpresses TNF-α in response to LPS (15), and dysregulated TNF-α production contributes to contractile dysfunction (11, 18, 24). NF-κB activity and TNF-α production are controlled by the molecular interaction between NF-κB and IκB-α (4). In the present study, myocardial IκB-α protein levels decreased 1–2 h after LPS treatment, returned to control level by 4 h, and increased above control level by 24 h. Coincidently with the decrease in myocardial IκB-α protein, NF-κB was activated and myocardial TNF-α levels increased. Cardiac contractile depression temporally followed these events. The results suggest that LPS causes a rapid degradation of myocardial IκB-α, followed by IκB-α resynthesis. It appears that myocardial IκB-α degradation and/or the level of this protein is critical to subsequent cardiac NF-κB activation and TNF-α synthesis.

The results of this study show that LPS treatment increases the steady-state levels of myocardial IκB-α protein. This increase in myocardial IκB-α coincided with the development of cardiac tolerance to subsequent endotoxemic contractile depression. Similarly, pretreatment with glucocorticoids, which induce cardiac functional tolerance to LPS, also increased myocardial IκB-α. Thus cardiac resistance to endotoxemic contractile depression is associated with an elevated myocardial IκB-α protein level. It remains unknown, however, whether the increase in myocardial IκB-α protein by either glucocorticoids or LPS is due to increased IκB-α gene transcription, RNA stability, translation rate, or protein stability.

Interestingly, myocardial IκB-α in LPS-pretreated animals remained similar to control level after subsequent LPS challenge. This preserved level of myocardial IκB-α may be due to a higher baseline level of IκB-α in LPS-pretreated animals or a combination of a higher baseline level with a decreased degradation rate. The time course utilized in this study is too
limited to address this issue. The role of IκB-α synthesis and stability in endotoxin tolerance has been examined by other investigators. In THP-1 cells, LPS tolerance is associated with a rapid regeneration of IκB-α (19). Both induction and stabilization of IκB-α have been observed in LPS-tolerant OVCAR-3 cells (16). Stabilization of IκB was related to inhibition of an inducible IκB-α kinase in the LPS-tolerant cells (16). It is likely that IκB-α stabilization is involved in maintaining myocardial IκB-α levels in the LPS-tolerant myocardium, although the mechanism responsible is unknown. An IκB kinase may possibly be downregulated in LPS-tolerant hearts. Alternatively, myocardial IκB-α may be stabilized by stress proteins, functioning as molecular chaperones, via direct protein-protein interaction (40). Indeed, we have observed an upregulation of heat shock protein 70 decreases LPS-stimulated NF-κB intranuclear translocation, suggesting an effect mediated through IκB (10).

The results of the present study indicate that myocardial LPS tolerance is associated with inhibition of NF-κB and myocardial TNF-α production. Other investigators have observed a similar attenuation in TNF-α production by LPS-tolerant cultured cells (12, 31, 38, 44) and a decrease in circulating TNF-α in LPS-tolerant animals (7, 32). Inhibition of NF-κB has been proposed as a potential mechanism of LPS tolerance (5, 17, 38, 44). The results generated by this study are in agreement with these previous findings and demonstrate that cardiac tolerance to LPS involves regulation of myocardial NF-κB activity and TNF-α production. An increased myocardial IκB-α protein level is also associated with glucocorticoid-mediated LPS tolerance. Glucocorticoids induce IκB-α in vitro and inhibit NF-κB-mediated proinflammatory cytokine production (2, 33). In this in vivo study, dexamethasone pretreatment increased myocardial IκB-α protein levels, inhibited myocardial TNF-α production, and preserved myocardial contractility. Thus inhibition of NF-κB-mediated myocardial TNF-α production by induction of myocardial IκB-α protein is a mechanism for cardiac tolerance to LPS. Regulation of IκB-α expression and/or stability has potential clinical application in controlling the inflammatory response. Further characterization of the mechanisms of myocardial IκB-α upregulation and/or stabilization may yield clinically accessible therapeutic strategies.

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REFERENCES


