Myocardial gene reprogramming associated with a cardiac cross-resistant state induced by LPS preconditioning

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Meng, Xianzhong, James M. Brown, Lihua Ao, Robert T. Rowland, Steven K. Nordeen, Anirban Banerjee, and Alden H. Harken . Myocardial gene reprogramming associated with a cardiac cross-resistant state induced by LPS preconditioning. *Am. J. Physiol.* 275 (*Cell Physiol.* 44): C475–C483, 1998.—Lipopolysaccharide (LPS) preconditioning induces cardiac resistance to subsequent LPS or ischemia. This study tested the hypothesis that resistance to LPS and resistance to ischemia are two manifestations of cardiac cross-resistance which may involve reprogramming of cardiac gene expression. Rats were preconditioned with a single dose of LPS (0.5 mg/kg ip). Cardiac resistance to LPS was examined with a subsequent LPS challenge. Cardiac resistance to ischemia was determined by subjecting hearts to ischemia-reperfusion. Total RNA was extracted from myocardium for Northern analysis of mRNAs encoding protooncoproteins, antioxidant enzymes, and contractile protein isoforms. Rats preconditioned with LPS 1–7 days earlier acquired cardiac resistance to endotoxemic depression. This resistance temporally correlated with resistance to ischemia. Pretreatment with cycloheximide (0.5 mg/kg ip) abolished resistance to both LPS and ischemia. LPS preconditioning induced the expression of c-*jun* and c-*fos* mRNAs. LPS also transiently increased mRNAs encoding catalase and Mn-containing superoxide dismutase. The expression of both α- and β-myosin heavy chain mRNAs was upregulated, whereas the expression of cardiac α-actin mRNA was suppressed. We conclude that *1*) LPS induces sustained cardiac resistance to both LPS and ischemia, *2*) resistance to ischemia and resistance to LPS seem to be two mechanistically indistinct components of cardiac cross-resistance, and *3*) the cardiac cross-resistance is associated with reprogramming of myocardial gene expression.

endotoxin; ischemia; protooncogenes; contractile protein isogenes; mRNA

ENDOTOXIN (lipopolysaccharide, LPS) exerts profound effects on the myocardium, leading to cardiac contractile depression and cardiac adaptation. We have reported that cardiac contractile dysfunction induced by sublethal LPS is reversible in the rat (25, 28) and that rat heart acquires resistance to subsequent LPS challenge after recovery from endotoxemic depression (28). Previous work from our laboratory (2) has also shown that rat heart acquires functional resistance to ischemia-reperfusion injury after LPS preconditioning. Further studies demonstrate that this cardiac resistance to ischemia is a delayed and sustained protective response, appearing at 24 h and persisting to 7 days after LPS preconditioning, and seems to involve de novo protein synthesis (24, 26, 31). It is likely that resistance to LPS and resistance to ischemia are two components of LPS-induced cardiac cross-resistance. However, it is unclear whether LPS-induced cardiac resistance to LPS is sustained and whether the resistance to LPS temporally correlates with the resistance to ischemia. Furthermore, it is unknown whether LPS-induced cardiac resistance to LPS is sensitive to protein synthesis inhibition as well. The temporal relation between these two cardiac protective responses and their individual sensitivity to protein synthesis inhibition are important to determine whether they are components of cardiac cross-resistance or mechanistically distinct.

The mechanisms underlying LPS-induced cardiac protective responses remain unknown. However, the delayed protection suggests that reprogramming of cardiac gene expression may be involved. Indeed, a variety of stressful stimuli can induce reprogramming of cardiac gene expression, leading to myocardial adaptation to a subsequent stress (8). LPS has numerous biological activities. LPS stimulates the production and release of cytokines by monocytes and macrophages and hence increases the levels of cytokines in circulation and in tissues including myocardium (19, 26). LPS also induces the expression of inducible nitric oxide (NO) synthase in the myocardium and thus increases cardiac NO level (20, 34). These secondary factors induced by LPS may regulate cardiac gene expression.

Indeed, LPS induces the in vivo expression of heat shock protein 70 (HSP70) in the interstitial cells of rat heart, and the cardiac resistance to LPS is accompanied by increased HSP70 in the myocardium (28). It is likely that HSP70 is involved in the cardiac resistance to LPS. However, upregulation of this stress protein may not be the only mechanism for LPS-induced myocardial protection because heat stress only provides partial cardiac resistance to LPS while eliciting more vigorous expression of cardiac HSP70 (28). Furthermore, cytokines, specifically tumor necrosis factor-α, seem not to be important contributors to LPS-induced cardiac functional resistance to ischemia (26). Possibly, cardiac cross-resistance to LPS and ischemia involves broader molecular adaptation than the expression of HSP70 and cytokines. Cardiac remodeling induced by stress involves the expression of protooncogenes (7) and fetal isogenes of contractile proteins, such as sarcomeric α-actin and myosin heavy chain (MHC) (18). The influence of LPS on the expression of protooncogenes and contractile protein isogenes in the myocardium remains to be determined. LPS has been shown to

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increase the activities of myocardial antioxidant enzymes, such as catalase, glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) (2, 21, 22). It is unclear whether the regulation of the activities of these enzymes by LPS involves the expression of cardiac genes encoding enzyme proteins.

The present study was undertaken *1*) to delineate the temporal relation between LPS-induced cardiac resistance to endotoxemic depression and cardiac resistance to ischemia; *2*) to examine the influence of protein synthesis inhibition on these two protective responses; *3*) to examine the expression of protooncogenes (c-*jun* and c-*fos*), antioxidant enzyme genes [Cu- and Zn-containing (Cu/Zn) SOD, Mn-containing SOD, catalase, and GSH-Px] and contractile protein isogenes (α-MHC, β-MHC, cardiac α-actin, and skeletal α-actin) in the myocardium after LPS preconditioning; and *4*) to delineate the relation between the expression of these genes with LPS-induced cardiac protective responses.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats, 300–325 g body wt, were purchased from Sasco (Omaha, NE). One hundred and sixteen animals were used in this study. The animals were acclimated in a quarantine room 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, University of Colorado Health Sciences Center. All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" [DHEW Publication no. (NIH) 85–23, revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205].

Chemicals and reagents. cDNAs complementary to mouse c-*fos*, mouse c-*jun*, human cardiac α-actin, rat cardiac MHC (both α- and β-isoforms), human Mn-SOD, and 28S rRNA were obtained from American Type Culture Collection (Rockville, MD). Rat catalase cDNA (clone pMJ-1010) was a generous gift from Dr. Shuichi Furuta, Shinshu University, Japan (12). Rat Cu/Zn-SOD cDNA (clone pGEM-32) was a generous gift from Dr. Linda Clerch, University of Miami (14). Rat GSH-Px cDNA (clone pBGPx-24) was a generous gift from Dr. Shinichi Yoshimura, Tokai University, Japan (39). Oligonucleotide probes to rat α-MHC and rat β-MHC were obtained from Oncogene Science (Uniondale, NY). Oligonucleotide probes to rat cardiac α-actin (GGGAGATGGGAGAGGGCCTCAGAGGATTCC, complementary to nucleotides 39–68 of 3′-untranslated region; Refs. 23, 40) and rat skeletal α-actin (AGAGAGAGCGCGTACACAGACGCGGTGCGC, complementary to nucleotides 1–30 of 3′-untranslated region; Ref. 40) were synthesized by the Department of Biochemistry, Colorado State University. Radioactive nucleotides were obtained from Du Pont-NEN Research Products (Boston, MA). T4 polynucleotide kinase, DNase, and DNA polymerase were obtained from New England Biolabs (Boston, MA). LPS (from *Salmonella typhimurium*), cycloheximide, and all other chemicals were obtained from Sigma Chemical (St. Louis, MO).

Experimental protocols. To examine the temporal relation between the cardiac resistance to endotoxemic depression and the cardiac resistance to ischemia, 48 rats were preconditioned with a single dose of LPS (0.5 mg/kg ip). A group of 24 preconditioned rats received a subsequent challenge with the same dose of LPS at 2 h or 1, 3, or 7 days after preconditioning ($n = 6$ at each time point). Hearts were isolated at 6 h after the second exposure to LPS, and cardiac contractility was assessed by the Langendorff technique and compared with saline control group (a single saline injection 6 h before heart isolation; $n = 10$) and single LPS group (a single LPS injection 6 h before heart isolation; $n = 10$). The time course of endotoxemic myocardial depression has been examined by our previous study (28). The maximal contractile depression is at 6 h after administration of this dose of LPS, and cardiac contractility is fully recovered at 24 h. Thus cardiac contractile depression was examined in this study at 6 h after administration of LPS. Another group of 24 preconditioned rats was killed, and hearts were isolated at 2 h or 1, 3, or 7 days after preconditioning ($n = 6$ at each time point). Isolated hearts were subjected to 25 min of normothermic global ischemia and 40 min of reperfusion. Postischemic functional recovery was compared with that of the saline control group (a single saline injection 2 h to 7 days before heart isolation; $n = 12$).

To examine the influence of protein synthesis inhibition on LPS preconditioning, 12 rats were pretreated with cycloheximide (0.5 mg/kg ip) 3 h before LPS preconditioning (0.5 mg/kg ip). These animals were divided into two groups at 3 days after LPS preconditioning. One group ($n = 6$) was used to examine the cardiac resistance to endotoxemic depression by subjecting to subsequent LPS challenge. Another group ($n = 6$) was used to examine the cardiac resistance to ischemia by subjection to isolated ischemia. Cycloheximide alone (0.5 mg/kg ip) was given to an additional group of 6 rats. Their hearts were isolated 3 days after cycloheximide treatment to examine the influence of this agent on baseline cardiac contractility and postischemic functional recovery. This cycloheximide dose has been demonstrated to abolish LPS-induced cardiac functional resistance to ischemia (26).

A group of 18 rats was treated with a single dose of LPS (0.5 mg/kg ip) and killed at 1, 2, 3, 6, or 12 h or 1, 2, 3, or 5 days ($n = 2$ at each time point) after the treatment. Hearts were rapidly excised and coronary vessels were flushed with 10 ml of cold (4°C) PBS (pH 7.4) by retrograde perfusion through the aortic root. Ventricular (both left and right) tissue was rapidly frozen in liquid nitrogen and stored at −70°C for RNA extraction.

Isolated heart perfusion and assessment of cardiac contractile function. Intrinsic cardiac contractility was determined by a modified isovolumetric Langendorff technique as described elsewhere (25, 28) and expressed as left ventricular developed pressure (LVDP). At 6 h after LPS challenge, beating hearts were rapidly excised into oxygenated Krebs-Henseleit solution containing (in mM) 5.5 glucose, 1.2 $CaCl_2$, 4.7 KCl, 25 $NaHCO_3$, 119 NaCl, 1.17 $MgSO_4$, and 1.18 KH_2PO_4. Normothermic retrograde perfusion was performed with the same solution in an isovolumetric and nonrecirculating mode. The perfusion buffer was saturated with a gas mixture of 92.5% O_2-7.5% CO_2 to achieve P_{O_2} of 450 mmHg, P_{CO_2} of 40 mmHg, and pH of 7.4. Perfusion pressure was maintained at 70 mmHg. A latex balloon was inserted through the left atrium into the left ventricle, and the balloon was filled with 0.15–0.20 ml of water to achieve a left ventricular end-diastolic pressure (LVEDP) of 5–10 mmHg (at peak and flat portion of LVEDP-LVDP curve). Pacing wires were fixed to the right atrium, and the heart was paced at 6.0 Hz. The myocardial temperature was maintained by placing the heart in an air-filled tissue chamber, which was kept at 37°C with circulating warm water. Hearts were perfused for 20 min, and LVDP was continuously recorded with a computerized pressure amplifier-digitizer (Maclab 8, AD Instrument, Cupertino, CA).

Global ischemia and reperfusion. The Langendorff technique for global ischemia and reperfusion has been described

elsewhere (24, 26, 27). Beating hearts were rapidly excised and arrested in cold Krebs-Henseleit solution. Normothermic retrograde perfusion was performed as mentioned above. A three-way stopcock was mounted above the aorta cannula to create global ischemia. After 15 min of perfusion (equilibration), hearts were subjected to 25 min of normothermic global ischemia, followed by 40 min of reperfusion. During ischemia, hearts were placed in a perfusate-filled organ bath chamber without pacing. The temperature of perfusate in the chamber was maintained at 37°C. LVDP and LVEDP were continuously recorded with the computerized pressure amplifier-digitizer.

RNA extraction and Northern analysis. Total RNA was extracted by the method of Chomczynski and Sacchi (4) with slight modification (27, 28). Gel electrophoresis and Northern blotting were carried out by using the methods previously described (27, 28). Briefly, ventricular tissue was homogenized in guanidinium thiocyanate solution, and total RNA was subsequently extracted with phenol and chloroform. RNA samples (12 μg) were size separated by electrophoresis on denatured 1% agarose gel and then transferred onto a nylon membrane using a vacuum transfer apparatus (Stratagene Cloning Systems, La Jolla, CA). Cross-linking was performed with a ultraviolet cross-linker (Stratagene Cloning Systems). Oligonucleotide probes were used to detect mRNAs encoding cardiac α-actin, skeletal α-actin, cardiac α-MHC, and cardiac β-MHC. The oligonucleotides were labeled with [γ-^{32}P]ATP by 5′-end labeling, and hybridization was performed overnight at 65°C. mRNAs encoding c-*jun*, c-*fos*, Cu/Zn-SOD, Mn-SOD, catalase, GSH-Px, total sarcomeric α-actin mRNA, total MHC mRNA, and 28S rRNA were detected with cDNA probes. Mouse c-*fos* and c-*jun* cDNAs and human cardiac α-actin, Mn-SOD, and 28S rRNA cDNAs were applied to hybridize rat mRNA species because these genes are evolutionarily conserved (6, 15, 23, 32). cDNA probes were labeled with [α-^{32}P]CTP by nick translation, and hybridization was performed overnight at 42°C. After hybridization, the membrane was washed with 0.3 M sodium chloride-0.3 M sodium citrate-0.1% SDS (pH 7.0) for 30 min at 65°C (for membranes probed with oligonucleotide) or 55°C (for membranes probed with cDNA) and then with 0.15 M sodium chloride-0.15 M sodium citrate-0.1% SDS (pH 7.0) for 10 min at room temperature. Autoradiography was accomplished with Kodak X-Omat film at −70°C. Densitometric measurement was carried out with a computerized laser densitometer (Molecular Dynamics, Sunnyvale, CA), and the density of each band of interest was normalized against its corresponding 28S rRNA band.

For each experiment, hybridization was performed on the same membrane by probing, stripping, and reprobing or on an identical membrane produced from the same gel.

Statistical analysis. Data are expressed as means ± SE. ANOVA was performed, and a difference was accepted as significant when $P < 0.05$ was verified by Bonferroni-Dunn post hoc analysis.

RESULTS

Temporal relation between cardiac resistance to LPS and cardiac resistance to ischemia. LVDP was 101 ± 3.6 mmHg in untreated rats. At 6 h after an exposure to LPS, LVDP was attenuated, whereas treatment with saline 6 h before heart isolation did not influence LVDP (Fig. 1). Hearts preconditioned with LPS 1, 3, or 7 days earlier were resistant to the myocardial depressive effect of subsequent LPS. LVDP in these groups was maintained after a subsequent LPS challenge (Fig. 1).

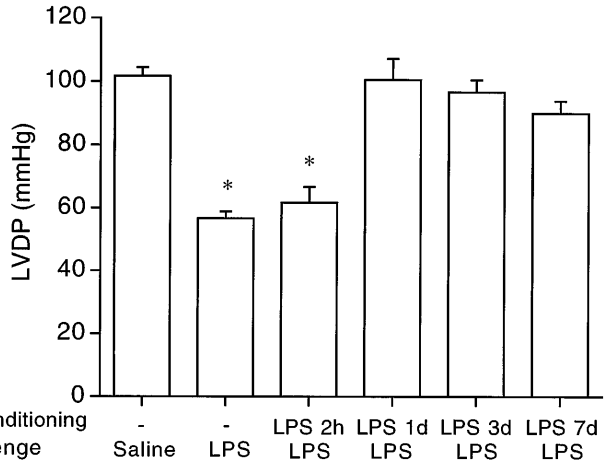

Fig. 1. Time course of lipopolysaccharide (LPS)-induced cardiac resistance to endotoxemic contractile depression. Rats were preconditioned with LPS (0.5 mg/kg ip) and exposed to LPS (0.5 mg/kg ip) again at 2 h or 1, 3, or 7 days (d) after preconditioning. Hearts were isolated at 6 h after the subsequent LPS challenge. Left ventricular developed pressure (LVDP) was assessed by the Langendorff method. Hearts preconditioned with LPS 1, 3, or 7 days earlier were resistant to a subsequent LPS challenge. Data are means ± SE; $n = 10$ in saline control and single LPS group and $n = 6$ in preconditioned groups. *$P < 0.01$ vs. saline control.

However, cardiac resistance to LPS was not present at 2 h after preconditioning.

LVDP declined, whereas LVEDP elevated in all hearts examined during ischemia (not shown). In saline control group, LVDP recovered to 45.1 ± 2.9 mmHg, and LVEDP remained at 49.6 ± 3.7 mmHg at the end of reperfusion. LPS preconditioning 1, 3, or 7 days before heart isolation significantly improved postischemic LVDP and LVEDP (Fig. 2, *A* and *B*). LPS preconditioning 2 h before heart isolation did not improve postischemic cardiac contractility or compliance.

Effect of cycloheximide pretreatment on LPS preconditioning. Administration of the protein synthesis inhibitor cycloheximide alone to rats did not affect baseline cardiac contractility (Fig. 3*A*) or postischemic cardiac functional recovery (Fig. 3*B*). However, cycloheximide pretreatment abolished the cardiac resistance to subsequent LPS observed at 3 days after LPS preconditioning. At 6 h after a subsequent LPS exposure, LVDP was 62.5 ± 7.2 mmHg in the group treated with cycloheximide plus LPS preconditioning (Fig. 3*A*), which was not different from the LVDP in the group treated with a single dose of LPS for 6 h (56.8 ± 2.5 mmHg). Similarly, pretreatment with cycloheximide abolished cardiac functional resistance to ischemia observed at 3 days after LPS preconditioning (Fig. 3*B*). LVDP in the group treated with cycloheximide plus LPS was 46.4 ± 4.4 mmHg ($P > 0.05$ vs. saline control) at the end of reperfusion, and LVEDP was 42.3 ± 2.8 mmHg ($P > 0.05$ vs. saline control) at the end of reperfusion.

Expressions of c-jun and c-fos mRNAs after LPS preconditioning. The results of Northern analysis showing c-*jun* and c-*fos* mRNAs are presented in Fig. 4. In the ventricular myocardium of saline-treated rats, c-*fos* mRNA was undetectable, whereas a low level of c-*jun* mRNA was detected. LPS induced bimodal expression

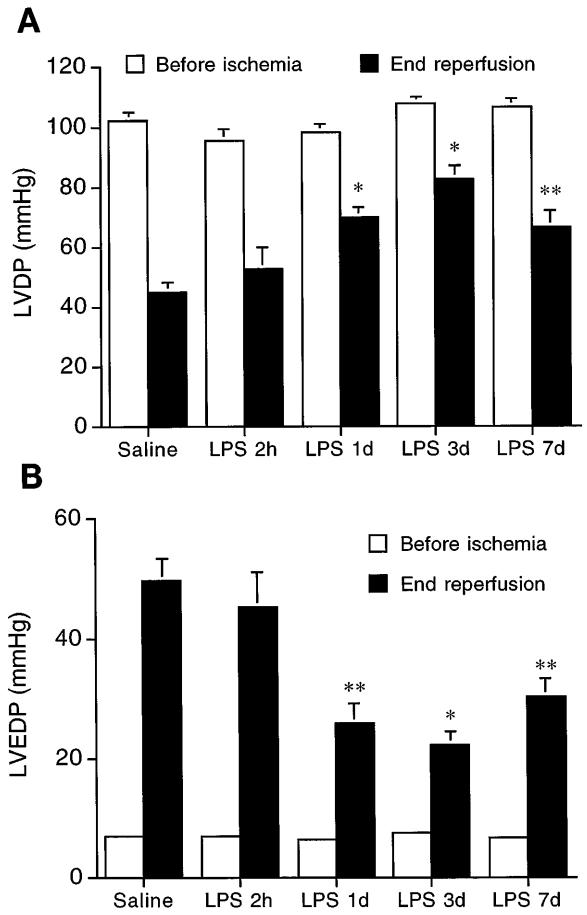

Fig. 2. Time course of LPS-induced cardiac functional resistance to ischemia. Rats were preconditioned with LPS (0.5 mg/kg ip). Hearts were isolated at 2 h or 1, 3, or 7 days after preconditioning and subjected to global ischemia-reperfusion (25:40 min). LVDP (*A*) and left ventricular end-diastolic pressure (LVEDP; *B*) were assessed by the Langendorff method before ischemia and at the end of reperfusion. LPS preconditioning 1, 3, or 7 days before heart isolation improved postischemic recovery of both LVDP and LVEDP. Data are means ± SE; $n = 12$ in saline control and $n = 6$ in LPS-preconditioned groups. *$P < 0.01$ vs. saline control; **$P < 0.05$ vs. saline control.

of c-*jun* mRNA in ventricular myocardium. The first peak (5.3-fold of saline control level) was observed at 1 h, and a second peak (8.7-fold of saline control level) manifested at 6 h after LPS treatment. The c-*jun* mRNA level was still elevated at 24 h. LPS also induced rapid expression of c-*fos* mRNA in ventricular myocardium; c-*fos* mRNA was detected at 1 h after LPS treatment. The c-*fos* mRNA level reached a peak (5.0-fold of saline control level) at 2 h, declined thereafter, and normalized at 24 h.

Expression of Cu/Zn-SOD, Mn-SOD, catalase, and GSH-Px mRNAs after LPS preconditioning. Figure 5 shows the results of Northern analysis of Cu/Zn-SOD, Mn-SOD, catalase, and GSH-Px mRNAs. All of these four mRNA species were expressed in ventricular myocardium of saline-treated heart. LPS treatment did not affect the levels of GSH-Px and Cu/Zn-SOD mRNAs. Catalase mRNA increased slightly at 6 h after LPS treatment (1.8-fold of saline control level). Mn-SOD mRNA increased primarily at 6 and 12 h (1.7- and 2.1-fold of saline control level) after LPS treatment, and an additional band with slightly bigger molecular size appeared.

Expression of MHC and α-actin isoform mRNAs after LPS preconditioning. The synthesized sarcomeric α-actin oligonucleotide probes have high selectivity. The cardiac α-actin oligonucleotide did not react with total RNA isolated from rat skeletal muscle but hybridized strongly with total RNA isolated from neonatal rat heart or adult rat heart (Fig. 6*A*). In contrast, the skeletal α-actin oligonucleotide hybridized strongly with total RNA isolated from rat skeletal muscle or neonatal

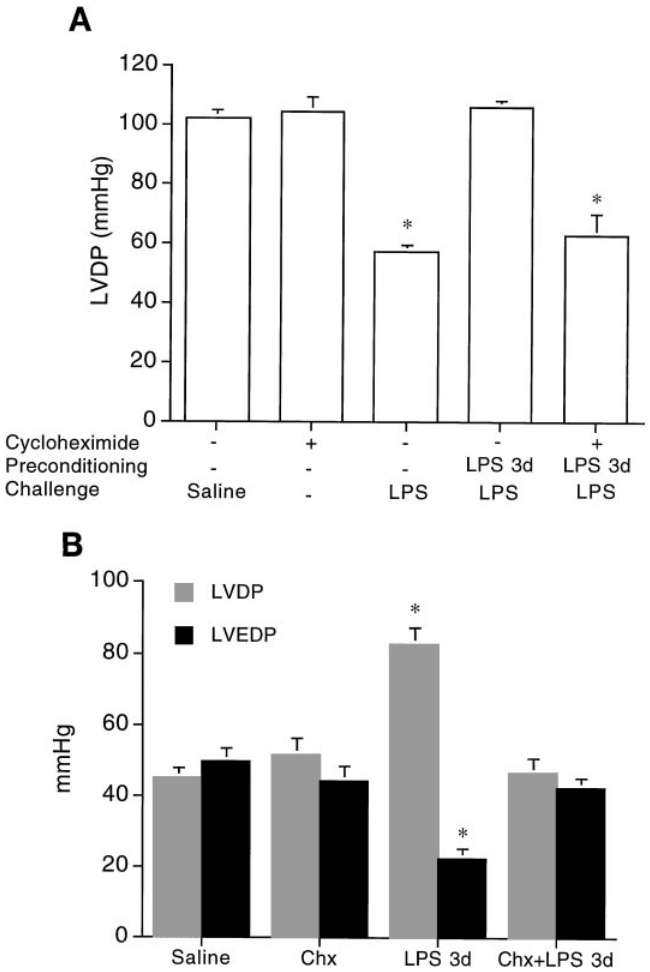

Fig. 3. *A*: effect of cycloheximide (Chx) pretreatment on cardiac resistance to subsequent LPS. Rats were treated with Chx (0.5 mg/kg ip, −3 h) before LPS preconditioning. Cardiac resistance to endotoxemic depression was determined at 3 days after preconditioning by challenging with LPS. LVDP was assessed by the Langendorff method at 6 h after subsequent LPS challenge. Chx pretreatment abolished the induced cardiac resistance to subsequent LPS. Data are means ± SE; $n = 10$ in saline control and single LPS group and $n = 6$ in other groups. *$P < 0.01$ vs. saline control. *B*: effect of Chx pretreatment on cardiac resistance to ischemia. Rats were treated with Chx (0.5 mg/kg ip, −3 h) before LPS preconditioning. Cardiac functional resistance to ischemia was determined at 3 days after preconditioning by subjecting isolated heart to global ischemia-reperfusion (25:40 min). LVDP and LVEDP were assessed by the Langendorff method at the end of reperfusion. Chx pretreatment prevented improvement of postischemic recovery of LVDP and LVEDP by LPS preconditioning. Data are means ± SE; $n = 12$ in saline control and $n = 6$ in other groups. *$P < 0.01$ vs. saline control.

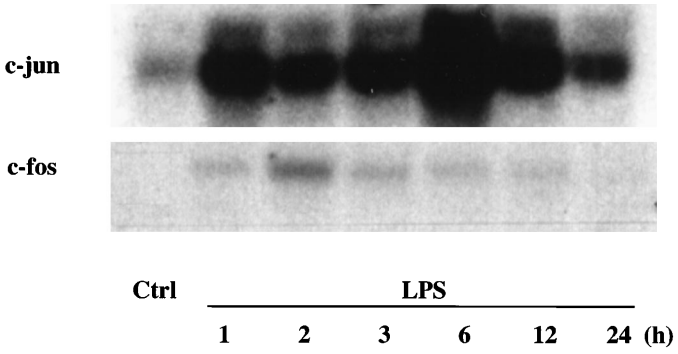

Fig. 4. Expression of c-*jun* and c-*fos* mRNAs in myocardium after LPS preconditioning. Rats were preconditioned with a single dose of LPS (0.5 mg/kg ip). Total RNA extracted from ventricular myocardium was size fractionated and blotted. Blots were probed with cDNAs complementary to c-*jun* and c-*fos* mRNAs. A representative Northern blot from 2 separate experiments shows increased c-*jun* and c-*fos* mRNAs in ventricular myocardium after LPS preconditioning. Ctrl, control.

rat heart (Fig. 6*A*). The results showing Northern analysis of mRNAs encoding MHC and α-actin isoforms are presented in Fig. 6, *B* and *C*. In the ventricular myocardium of saline-treated rats, α-MHC, β-MHC, cardiac α-actin, and skeletal α-actin mRNAs were constitutively expressed. LPS treatment resulted in differential upregulation of α-MHC and β-MHC mRNAs in ventricular myocardium. α-MHC mRNA increased primarily at 3–12 h, peaked at 3 h (2.4-fold of saline control level), and normalized at 24 h after LPS treatment. β-MHC mRNA increased at 2 h after LPS treatment and peaked at 24 h (5.7-fold of saline control level; Fig. 6*B*). An increase in total MHC mRNAs at 2–24 h was also detected when the blot was probed with rat cardiac MHC cDNA which hybridizes with both α- and β-isoforms of MHC mRNA (Fig. 6*B*). β-MHC mRNA remained elevated at 3 days and normalized at 5 days after LPS treatment (Fig. 6*C*). LPS treatment had minimal influence on skeletal α-actin mRNA level in ventricular myocardium. However, cardiac α-actin mRNA decreased at 6 h after LPS treatment and declined to 20% of saline control level by 24 h (Fig. 6*B*). The full-length cardiac α-actin cDNA recognizes both cardiac α-actin mRNA and skeletal α-actin mRNA. It may also cross-react with β-actin mRNA, since an additional band with slightly bigger molecular size was detected when the blot was probed with this cDNA. Total α-actin mRNA decreased in a temporal pattern similar to that of cardiac α-actin mRNA (Fig. 6*B*), and total α-actin mRNA remained slightly lower at 5 days, although it was recovering at 3 and 5 days after LPS treatment (Fig. 6*C*).

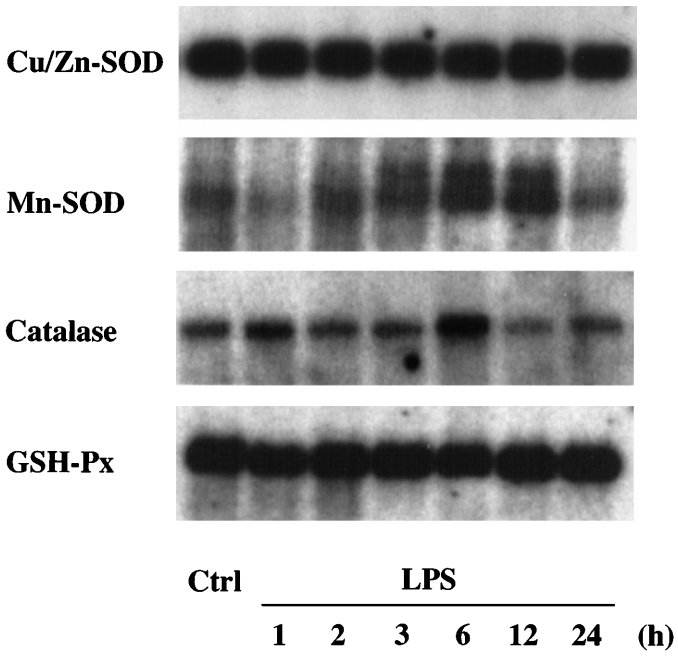

Fig. 5. Expression of mRNAs encoding antioxidant enzymes in myocardium after LPS preconditioning. Rats were preconditioned with a single dose of LPS (0.5 mg/kg ip). Total RNA extracted from ventricular myocardium was size fractionated and blotted. Blots were probed with cDNAs complementary to Cu- and Zn-containing superoxide dismutase (Cu/Zn-SOD), Mn-containing SOD, catalase, and glutathione peroxidase (GSH-Px) mRNAs. A representative Northern blot from 2 separate experiments shows that Mn-SOD and catalase mRNAs increased in ventricular myocardium after LPS preconditioning, whereas Cu/Zn-SOD and GSH-Px mRNAs were unchanged.

DISCUSSION

This study demonstrates that LPS-induced cardiac functional resistance to endotoxemic depression and cardiac functional resistance to ischemia are durable (lasting for days) cardioprotective responses. Cardiac resistance to subsequent LPS and cardiac resistance to ischemia appear to be allied components of LPS-induced cardiac cross-resistance because the two cardioprotective responses are temporally correlated and both are sensitive to protein synthesis inhibition. The development of this cardiac cross-resistance is associated with reprogramming of the expression of cardiac genes encoding protooncoproteins, antioxidant enzymes, and contractile protein isoforms.

Our previous studies (25, 28) have shown that sublethal LPS induces delayed and reversible cardiac contractile dysfunction in the rat. Cardiac contractile dysfunction occurs at 4 h after an exposure to LPS, becomes maximal at 6 h, and is completely recovered at 24 h. Interestingly, rat heart acquires resistance to subsequent LPS challenge after its recovery from endotoxemic depression, and this resistance is manifested as a lack of cardiac contractile dysfunction after a subsequent LPS exposure (28). However, the mechanisms underlying this cardiac resistance remain unknown.

Systemic LPS tolerance is well recognized. Tolerance develops after repeated sublethal doses of LPS and is characterized by an attenuated response to a subsequent LPS challenge. Animals rendered tolerant are resistant to systemic LPS toxicosis in terms of proinflammatory cytokine production (41), pyrogenesis (33), and mortality (11). The results of the present study confirm that LPS tolerance develops in the heart after LPS preconditioning (28). The development of cardiac resistance to LPS is time dependent. The cardiac

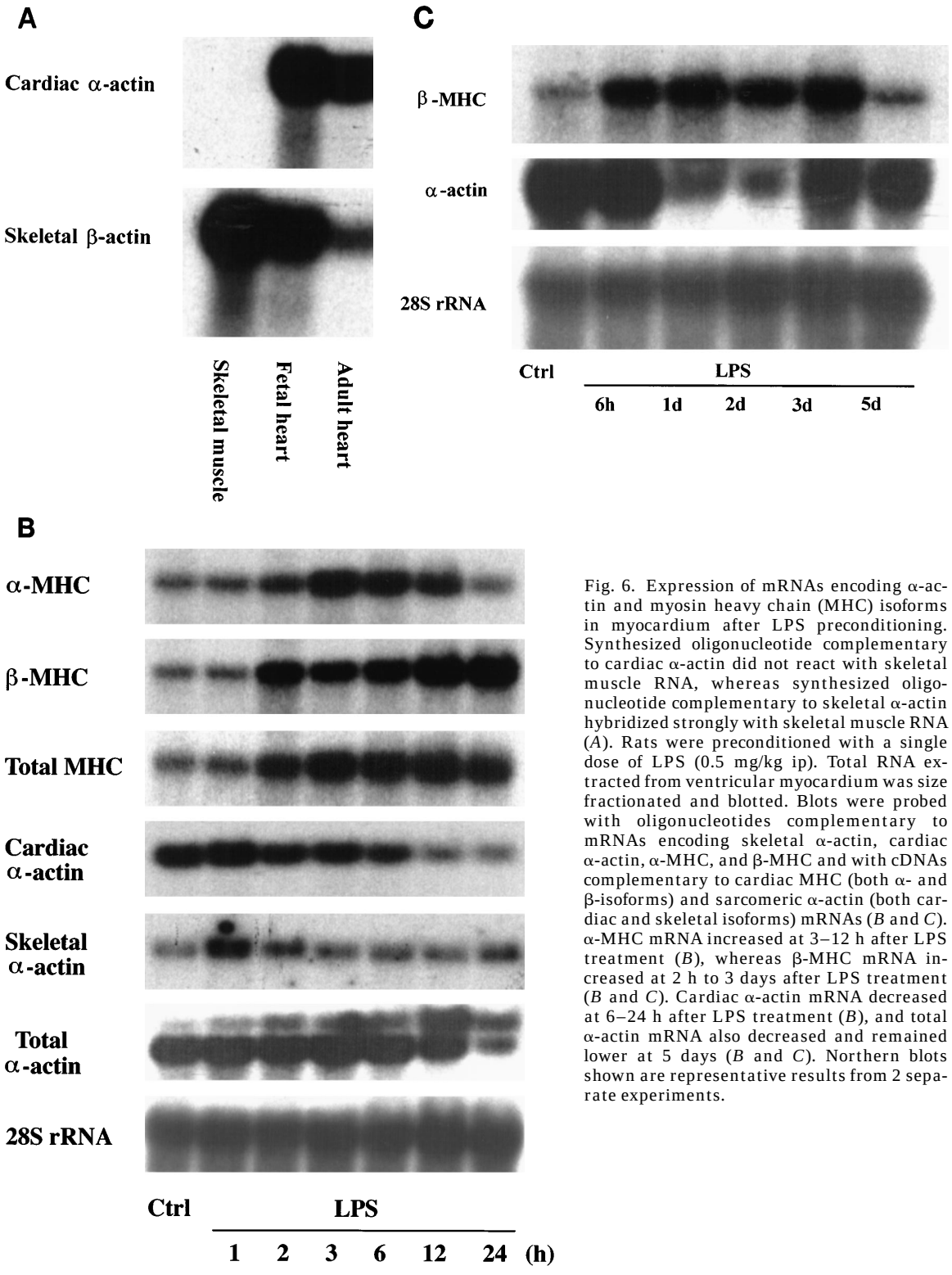

Fig. 6. Expression of mRNAs encoding α-actin and myosin heavy chain (MHC) isoforms in myocardium after LPS preconditioning. Synthesized oligonucleotide complementary to cardiac α-actin did not react with skeletal muscle RNA, whereas synthesized oligonucleotide complementary to skeletal α-actin hybridized strongly with skeletal muscle RNA (*A*). Rats were preconditioned with a single dose of LPS (0.5 mg/kg ip). Total RNA extracted from ventricular myocardium was size fractionated and blotted. Blots were probed with oligonucleotides complementary to mRNAs encoding skeletal α-actin, cardiac α-actin, α-MHC, and β-MHC and with cDNAs complementary to cardiac MHC (both α- and β-isoforms) and sarcomeric α-actin (both cardiac and skeletal isoforms) mRNAs (*B* and *C*). α-MHC mRNA increased at 3–12 h after LPS treatment (*B*), whereas β-MHC mRNA increased at 2 h to 3 days after LPS treatment (*B* and *C*). Cardiac α-actin mRNA decreased at 6–24 h after LPS treatment (*B*), and total α-actin mRNA also decreased and remained lower at 5 days (*B* and *C*). Northern blots shown are representative results from 2 separate experiments.

resistance to endotoxemic contractile depression developed at 24 h and persisted to 7 days after LPS preconditioning. However, the resistance was not induced at 2 h. This time course correlates well with the cardiac resistance to ischemia, which occurs at 24 h and lasts up to 7 days after LPS preconditioning. Furthermore, both resistance to LPS and resistance to ischemia were abolished by protein synthesis inhibition. The results suggest that resistance to LPS and resistance to ischemia after LPS preconditioning are components of LPS-induced cardiac cross-resistance. Thus LPS preconditioning induces a delayed and sustained cardiac cross-resistant state that appears to obligate the synthesis of protective proteins.

The mechanisms of systemic LPS tolerance remain unclear. Several hypotheses have been formulated, including *1*) suppression of immunoresponse to LPS by endogenous glucocorticoids (42), *2*) reduced production of proinflammatory cytokines by monocytes and macrophages (37), and *3*) elevated NO production in macrophages (10). Although all of these mechanisms may indirectly contribute to the attenuated cardiac response to LPS, here we have investigated the myocardial endogenous mechanisms, i.e., myocardial adaptation, in the elaboration of LPS-induced cardiac cross-resistance. We hypothesized that LPS preconditioning induces nonspecific myocardial adaptation resulting from reprogramming of cardiac gene expression and thus renders the heart resistant to subsequent LPS, ischemia, and perhaps other noxious stimuli.

We have reported that LPS preconditioning induces HSP70 in myocardial interstitial cells of rat heart (28). HSP70 has been shown to inhibit tumor necrosis factor-α production by LPS-stimulated monocytes or macrophages (35). Indeed, cardiac HSP70 protects myocardium against ischemic injury (30) and may be involved in cardiac resistance to LPS (28). However, heat stress only provides partial cardiac resistance to LPS, although it elicits more vigorous expression of cardiac HSP70 (28). Thus LPS-induced cardiac cross-resistance may not be explained exclusively by the induction of HSP70. This cross-resistance may involve broader molecular adaptation. In this regard, Das et al. (8) have proposed that myocardial adaptation to stress may involve the expression of several group genes including protooncogenes, stress protein genes, and antioxidant enzyme genes. In the present study, we examined the expression of protooncogenes (c-*jun* and c-*fos*) and antioxidant enzyme genes (Cu/Zn-SOD, Mn-SOD, catalase, and GSH-Px). We also examined the expression of MHC and α-actin isogenes (α-MHC, β-MHC, cardiac α-actin, and skeletal α-actin) because the expression of these genes has been shown to be associated with myocardial adaptation induced by hemodynamic stress (18).

Both c-*jun* and c-*fos* are components of transcription factor AP-1, which regulates the transcription of numerous cardiac genes. It is unknown whether LPS influences cardiac c-*jun* and c-*fos* gene expression. By Northern analysis, we noted that LPS preconditioning induced a rapid but transient increase in c-*jun* and c-*fos* mRNAs in ventricular myocardium. The transcripts of these two protooncogenes increased at 1 and 2 h after LPS treatment. Interestingly, the expression of c-*jun* mRNA was temporally bimodal. The first peak was at 1 h, and a second peak manifested at 6 h. Indeed, different forms of stress upregulate the expression of c-*jun* and c-*fos* in mammalian cells (7, 18, 27, 36, 38). The rapid expression of c-*jun* and c-*fos* mRNAs may be the result of acute systemic and/or cardiac stress after administration of LPS. It is likely that the second phase of c-*jun* expression is induced by secondary factors, such as cytokines, or by myocardial depression itself. If the latter is true, the second phase expression of c-*jun* mRNA may serve as a marker of myocardial depression.

The activation of AP-1 and expression of fetal isoforms of α-actin and MHC indicate cardiac fetal reprogramming (18). In the ventricular tissue of saline-treated adult rat heart, cardiac α-actin, skeletal α-actin, α-MHC, and β-MHC mRNAs were constitutively expressed. LPS upregulated the expression of both α-MHC and β-MHC mRNAs in the ventricular tissue. However, changes in the levels of these two gene transcripts were temporally divergent. α-MHC mRNA increased transiently (2–12 h), whereas the increase in β-MHC mRNA was sustained. β-MHC mRNA level was maximal at 24 h after LPS treatment when cardiac cross-resistance developed and remained elevated at 3 days. LPS did not affect the level of skeletal α-actin mRNA in the ventricular myocardium. However, the expression of cardiac α-actin mRNA was depressed, and cardiac α-actin mRNA decreased to 20% of control level at 24 h after LPS treatment. Although the significance of the differential expression of α-actin and MHC isogenes, i.e., downregulation of cardiac α-actin mRNA expression and upregulation of β-MHC mRNA expression, is not immediately known from this study, this gene program is distinct from the gene program exhibited in cardiac hypertrophy (18) and may be specific to LPS-induced myocardial adaptation.

Free radicals contribute to ischemia-reperfusion injury (3) and have recently been implicated in LPS-induced organ dysfunction (9). Few studies have examined the influence of LPS on antioxidant enzyme gene transcription in tissues (5, 13). Clerch et al. (5) probed mRNAs encoding antioxidant enzymes in rat lung at 1–6 h after LPS treatment. Mn-SOD mRNA increased, whereas catalase mRNA decreased in the early phase after LPS treatment. Cu/Zn-SOD and GSH-Px mRNAs were unchanged. Ghosh et al. (13) examined antioxidant enzyme gene expression in rat heart, liver, and kidney at 12 and 24 h after treatment with LPS at doses similar to those utilized in the present study. In the heart, Cu/Zn-SOD mRNA decreased at both time points, whereas Mn-SOD and catalase mRNAs increased at 24 h. These investigators (13) also noted that alterations in antioxidant enzyme mRNAs varied with the tissue type and dose of endotoxin examined. LPS preconditioning has been reported to increase antioxidant enzyme (catalase, SOD, and GSH-Px) activities in the myocardium (2, 21, 22). It is unclear from previous studies whether increased myocardial antioxidant enzyme activities are due to enhanced expression of cardiac genes encoding enzyme proteins.

Using Northern analysis at a broader time range, we examined the influence of LPS preconditioning on cardiac mRNAs encoding antioxidant enzymes. Catalase mRNA was increased transiently at 6 h after LPS preconditioning, whereas GSH-Px and Cu/Zn-SOD mRNAs were unchanged. An obvious change is that Mn-SOD mRNA increased at 6 and 12 h after LPS preconditioning. This time course is slightly different from the previous observation by Ghosh et al. (13) that Mn-SOD mRNA increased in the rat heart at 24 h after

LPS treatment. It should be pointed out that, in the present study, mRNA level was analyzed in ventricular myocardium rather than in the whole heart. There may be location (ventricle vs. atrium) differences in the expression of myocardial antioxidant enzyme genes. Furthermore, cardiac vessels were thoroughly flushed before collection of the myocardium in the present study. Thus the potential contribution of blood cells was avoided. These differences in sampling may account for the difference between our finding and the previous report (13). An additional band with slightly bigger molecular size also appeared at 6 and 12 h after LPS preconditioning. The Mn-SOD mRNA band of bigger size may be derived by utilization of different transcription termination sites of the Mn-SOD gene. In this regard, Hurt et al. (17) have demonstrated that rat Mn-SOD gene can generate multiple mRNA species due to alternate polyadenylation. Ho et al. (16) have detected six species of Mn-SOD mRNA, ranging from 1.3 to 4.2 kb, in hyperoxic rat lungs. In view of previous studies (21, 22) and the current findings, it is likely that the increase in catalase activity and Mn-SOD activity in the LPS-preconditioned myocardium is, at least partially, due to increased gene transcription. The increase in GSH-Px activity and Cu/Zn-SOD activity may be regulated at posttranscriptional levels.

Taken together, LPS preconditioning increases c-*jun*, c-*fos*, α-MHC, β-MHC, catalase, and Mn-SOD mRNAs and decreases cardiac α-actin mRNA in the myocardium. This broad program of molecular remodeling may play a central role in the LPS-induced cardiac cross-resistance. Fetal isoform of MHC utilizes ATP more efficiently for contractile function (1, 29). The increased expression of β-MHC may lead to phenotypic changes in the myocardium, resulting in enhanced cardiac resistance to a subsequent insult that disrupts myocardial energy metabolism. Catalase and Mn-SOD are key members of the anti-free radical defense and play important roles in the protection against ischemia-reperfusion injury (21, 22). Moreover, LPS preconditioning induces the expression of HSP70 in the myocardium (28). HSP70 is involved in processing newly synthesized cellular proteins and may play an important role in renaturing denatured proteins. Together, these molecular remodeling events may promote the cardiac cross-resistance induced by LPS preconditioning.

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