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Cardioprotective Effects of Low Dose Bacterial Lipopolysaccharide May Not Be Directly Associated with Prostacyclin Production

Chang-Hyun Moon, Ji Young Kim, Soo Hwan Lee, and Eun Joo Baik

Department of Physiology, Scool of Medicine, Ajou University, Suwon 442-749, Korea

Sublethal dose of bacterial lipopolysaccharide (LPS) would induce protection against cardiac ischemic/reperfusion (I/R) injury. This study examines the following areas: 1) the temporal induction of the cardioprotection produced by LPS; and 2) the relations between a degree of protection and the myocardial prostacyclin (PGI_2) production. Rats were administered LPS (2 mg/kg, i.v.), and hearts were removed 1, 4, 8, 14, 24, 48, 72,and 96 h later. Using Langendorff apparatus, haemodynamic differences during 25 min of global ischemia/30 min reperfusion were investigated. The concentration of PGI_2 in aliquots of the coronary effluent was determined by radioimmunoassay as its stable hydrolysis product 6-keto-$PGF1_\alpha$ and lactate dehydrogenase release were measured as an indicative of cellular injury. LPS-induced cardiac protection against I/R injury appeared 4 h after LPS treatment and remained until 96 h after treatment. PGI_2 release increased 2-3 fold at the beginning of reperfusion compared to basal level except in hearts treated with LPS for 48 and 72 h. In hearts removed 48 and 72 h after LPS treatment, basal PGI_2 was increased. To determine the enzymatic step in relation to LPS-induced basal PGI_2 production, we examined prostaglandin H synthase (PGHS) protein expression, a rate limiting enzyme of prostaglandin production, by using Western blot analysis. LPS increased PGHS protein expression in hearts at 24, 48, 72, 96 h after LPS treatment. Induction of PGHS expression appeared in both isotypes of PGHS, a constitutive PGHS-1 and an inducible PGHS-2. To identify the correlationship between PGI_2 production and the cardioprotective effect against I/R injury, indomethacin was administered *in vivo* or *in vitro*. Indomethacin did not inhibit LPS-induced cardioprotection, which was not affected by the duration of LPS treatment. Taken together, our results suggest that PGI_2 might not be the major endogenous mediator of LPS-induced cardioprotection.

Key Words: Bacterial lipopolysaccharide, Cardioprotection, Ischemia, Reperfusion, Heart, Prostacyclin

INTRODUCTION

High doses of endotoxin or bacterial lipopolysaccharide (LPS) promote in vivo tissue injury by inducing an uncontrolled inflammatory response (Brown et al, 1990). In contrast, low doses of endotoxin can induce tolerance against ensuing tissue injury (Frank et al, 1980) and cardioprotective responses to endotoxin have occurred. In one of the first studies to show a cardioprotective effect of endotoxin or one of its derivatives, Brown et al (1989a) found that pretreatment with a non-lethal dose of endotoxin for 24 hours resulted in an improvement in the recovery of ventricular function in isolated Langendorff-perfused rat hearts subjected to global ischemia and reperfusion. A sublethal dose of endotoxin given 36 h (Bensard et al, 1990) prior to cardiac ischemia and reperfusion improves myocardial functional recovery in isolated, perfused rat hearts. In addition, pretreatment with more distal cytokine mediators of endotoxin activity, such as tumor necrosis factor (Brown et al, 1992) and interleukin-1 (Brown et al, 1989b) also protects the heart from ischemic injury. The mechanisms and time course, however, have not been

Corresponding to: Chang-Hyun Moon, Department of Physiology, School of Medicine, Ajou University, Suwon 442-749, Korea. (Tel) 0331-219-5041, (Fax) 0331-219-5049

fully identified.

Prostacyclin (PGI_2) is the major active arachidonic acid metabolite produced by vascular endothelium and myocardium (Moncada et al, 1976; 1977). It is the most potent endogenous inhibitor of platelet aggregation ever discovered (Moncada et al, 1976). It is also a potent systemic vasodilator (Fitzpatrick et al, 1978) and coronary artery vasodilator (Raz et al, 1977). PGI_2 has been demonstrated to be protective in a number of experimental models of myocardial ischemia (Ogletree et al, 1979; Araki & Lefer, 1980; Jugdutt et al, 1981; Ribeiro et al, 1981). Lefer et al demonstrated the usefulness of PGI_2 for the treatment of acute myocardial ischemia (Lefer et al, 1978). This was based on the ability of PGI_2 to inhibit platelet aggregation and favorably alter myocardial oxygen supply and demand by increasing coronary artery blood flow and reducing systemic blood pressure. Melin & Becker (1983) demonstrated that PGI_2 protected the heart from ischemic injury without increasing collateral blood flow. It was also reported that PGI_2 inhibits in vitro production of cytotoxic oxygen-free radicals and release of degradative lysosomal enzymes (Fantone & Kinnes, 1983; Fantone et al, 1984a; Fantone et al, 1984b). Since LPS stimulates the synthesis of arachidonic acid metabolites in a number of cell types including endothelial cells, neutrophils, glomerular mesangial cells, macrophages, and monocytes (Cybulsky et al, 1988), we hypothesize that the cytoprotective effect of LPS in myocardial ischemia and reperfusion is at least partly due to the PGI_2 production.

The purpose of this study, therefore, was to develop an understanding of tolerance acquisition and to extend the time period of this protection. Therefore, this study was designed to examine following areas: 1) the temporal induction of the cardioprotection produced by endotoxin, and 2) the relations between a degree of protection and the myocardial PGI_2 production.

METHODS

Animals and pretreatment with endotoxin

Male Sprague-Dawley rats with an average weight of 300~350 g were used. They were housed on a 12 h light/12 h dark photoperiod and were allowed free access to standard rat chow and water. Rats had given endotoxin derived from Escherichia coli (0111 : B4, Sigma, 2 mg/kg) or an equivalent volume of saline by intravenous injection at indicated time before the isolation of heart.

Preparation of isolated rat hearts

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and heparinized (500 units, i.v.). Hearts were rapidly excised and then perfused (70 mmHg) in retrograde fashion at the aortic root with a Krebs-Henseleit solution containing 1.2 mM Ca^{2+}, 4.7 mM KCl, and 25.0 mM $NaHCO_3$ on a Langendorff apparatus. A gas mixture (95% O_2/5% CO_2) at pH of 7.4 was used. A saline-filled latex baloon was placed through the left atrium into the left ventricle and adjusted to a left ventricular end diastolic pressure of 10 mmHg. After 15 min of equilibration, left ventricular developed pressure (LVDP), positive (+dP/dt) and negative (-dP/dt) first derivatives (measures of contractility and relaxation rate, respectively). Heart rate was recorded by using direct pressrue and differentiator amplifiers (Grass Instrument Co.). Coronary flow rate was determined by the collection of coronary effluent for 1 min. Subsequently, hearts were subjected to 25 min of global normothermic (37°C waterbath) ischemia (aortic root stopcock) and 30 min of reperfusion. In some experiments, isolated hearts were infused with indomethacin (1 μmole/l) or vehicle (Na_2CO_3, 10 μM) for 30 min before ischemia. Samples of coronary eluent were collected to determine lactate dehydrogenase by an optimised spectrophotometric assay (LDL-10, Sigma).

Measurement of PGI2 release

PGI_2 levels were measured in aliquots of the coronary effluent at various time periods during perfusion, as shown in the results. PGI_2 was measured by radioimmunoassay procedure and antisera given by Dr. Daniel Hwang of the Pennington Biomedical Research Center, Lousiana Stated University. The results presented in this report are for 6-keto-PGF1a, the stable hydrolysis product of PGI_2, which is the most abundant cardiac prostaglandin in animal and human tissue (De Deckere et al, 1977; Brandt et al, 1984).

Determination of myocardial prostaglandin H synthase (PGHS) content by Western blot analysis

The levels of prostaglandin H synthase (PGHS)-1 and PGHS-2 were analyzed by using a Western blot analysis method. Frozen left ventricular tissues were homogenized in PBS (pH7.4) containing 10 mM EDTA, 5 mM EGTA, 1 mM PMSF, and 10 uM leupeptin. Microsomes from the lysed cells were electrophoresed on an 10% SDS-polyacrylamide gel and transblotted onto a polyvinylidene difluride membrane (Millipore). The blot was blocked with 5% nonfat dry milk, incubated in the antibody solution overnight, and treated with goat antirabbit IgG, conjugated with alkaline phosphatase. Color development was made with alkaline phosphatase color reagents (Sigma Co.) containing 5-bromo-2-chloro-3-indolyphosphate and nitroblue tetrazolium in 0.1 M Tris buffer. In addition to the molecular weight markers (Bio-Rad Laboratories), ram seminal vesicle PGHS was used as a standard for PGHS-1.

Statistical analyses

The data were expressed in mean±SE and evaluated for statistical significance by analysis of variance (ANOVA). A p value of less than 0.05 was considered significant.

RESULTS

Effect of LPS treatment on cardiac function

Isolated perfused hearts from rats exposed to LPS (2 mg/kg, i.v.) for 4 and 8 h before experiment showed greatly depressed contractile activity when compared with control as shown in Fig. 1. LVDP of LPS-treated hearts for 4 and 8 h was only 24.6 % and 32.0 % of that of control (17.8±1.5 and 23.2±

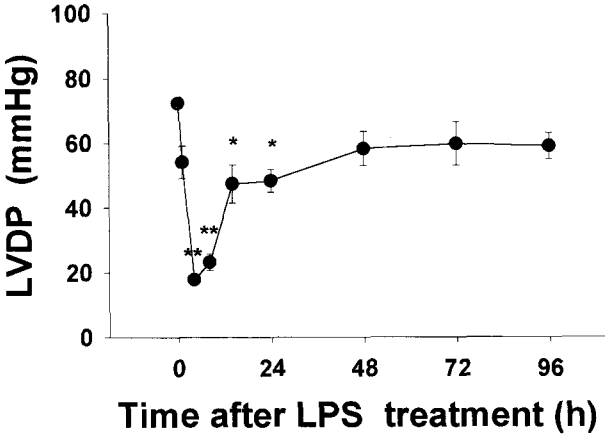

Fig. 1. Effects of LPS on basal left ventricular developed pressure (LVDP). Rats were pretreated with LPS (2 mg/kg) for each time period (n=8-9 rats). Hearts were rapidly excised and then perfused in retrograde fashion on a Langendorff apparatus. Basal LVDP was determined after equilibration for 15 min. *p<0.05, **p<0.01 vs control (LPS 0 h).

Table 1. Effects of LPS on haemodynamic parameters in isolated rat hearts

	Heart Rate(beats/min)		LVEDP(mmHg)		CFR(ml/min)		TTC(min)[a]
	Preischemia	Post I/R	Preischemia	Post I/R	Preischemia	Post I/R	
LPS 0h	324.3±7.5	287.9±8.5	8.6±6.3	53.9±6.3	15.4±0.8	9.2±0.6	17.2±0.5
LPS 1h	320.5±5.3	252.3±23.3	5.5±0.8	41.1±9.2	15.5±1.6	7.7±1.6	18.7±1.2
LPS 4h	325.7±7.3	284.8±6.2	7.8±0.4	34.3±7.2	10.7±0.8	9.5±0.7	20.8±0.5*
LPS 8h	313.9±16.9	290.5±9.5	8.9±0.6	20.3±4.5**	22.2±5.4	8.3±0.6	17.7±0.5
LPS 14h	345.8±15.3	306.8±8.6	8.1±0.8	23.9±4.1**	15.0±2.4	9.2±0.5	19.6±0.9*
LPS 24h	353.4±10.1	310.2±7.8	6.9±0.6	26.1±4.7*	14.7±1.3	9.4±0.9	19.2±0.6*
LPS 48h	327.0±6.8	285.0±6.6	8.0±0.8	38.4±3.3	13.4±1.3	9.4±1.8	18.4±0.8
LPS 72h	320.4±15.9	283.3±9.7	6.9±0.6	29.6±4.5	13.8±1.5	10.5±1.3	20.0±0.8*
LPS 96h	305.1±10.9	228.6±17.8	6.8±0.6	42.4±7.1	13.8±0.8	10.7±1.6	19.4±0.4*

*p<0.05, **p<0.01 vs control(LPS 0h)
[a]Time to contracture as ischemic time producing 5 mmHg increase in LVDP.

2.6 vs 72.4±1.8 mmHg). Contractility gradually returned toward control values at subsequent time periods, especially at 48, 72, and 96 h (58.3±5.3, 59.8±6.7 and 59.1±4.1 mmHg, respectively). Similar results were obtained in ±dP/dt (data not shown). Other haemodynamic parameters such as heart rate (h), left ventricular end diastolic pressure (LVEDP) and coronary flow rate (CFR) did not change significantly by LPS treatment as summarized in Table 1.

Temporal effects of LPS on cardiac function after ischemia and reperfusion

After ischemia for 25 min and reperfusion for 30 min, hearts from control rats showed decreased LVDP, +dP/dt, -dP/dt and DP (42.59±2.01, 51.52±5.52, 50.82±1.30 and 36.48±5.90 %, repectively) compared to that of basal (preischemic) state (Fig. 2). In contrast, after ischemia and reperfusion, hearts from rats pretreated with LPS 4, 8, 14, 24, 48, 72, or 96 h before isolation showed increased LVDP, +dP/dt, -dP/dt and DP compared to hearts from saline-treated control rats. Hearts treated with LPS 1 h before, registered no significant changes in contractile function after ischemia and reperfusion. Other haemodynamic parameters such as heart rate (HR) and coronary flow rate (CFR) after ischemia and reperfusion did not change significantly by LPS treatment, except that left ventricular end diastolic pressure (LVEDP) decreased in hearts treated with LPS

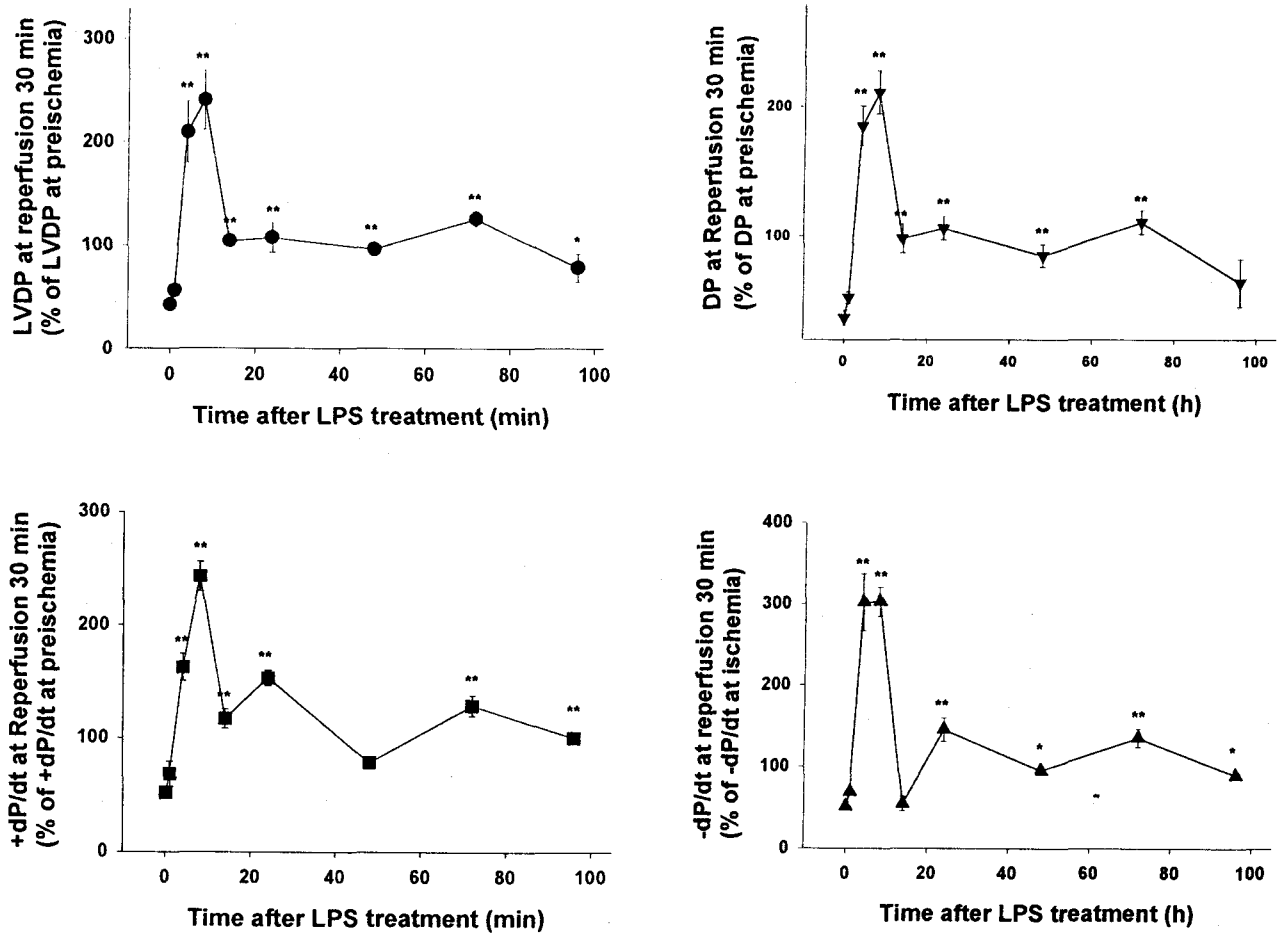

Fig. 2. Effects of LPS on ventricular function of isolated hearts after global normothermic ischemia and reperfusion. Isolated hearts from rats treated with LPS during each time period were equilibrated for 15 min. Before and after 25 min-ischemia/ 30 min-reperfusion (I/R), hemodynamic parameters were determined. Data were expressed as a percentage of the level of each parameter at preischemic state, which meaned cardiac recovery of each parameter against I/R injury (n=8-9 rats). A, left ventricular developed pressure (LVDP); B, double product (DP=LVDP x heart rate /1000); C, +dP/dt; D, -dP/dt. *$p<0.05$, **$p<0.01$ vs control (LPS 0 h)

8, 14, 24, and 72 h before. The results are summarized in Table 1. Time to contracture after ischemic insult (TTC) was increased in hearts treated LPS for 4, 14, 24, 72, and 96 h. The release of LDH from coronary effluent after ischemia/reperfusion (I/R) insult was decreased by LPS treatment, especially in hearts treated with LPS for 8, 14, 24, 48 h (Fig. 3).

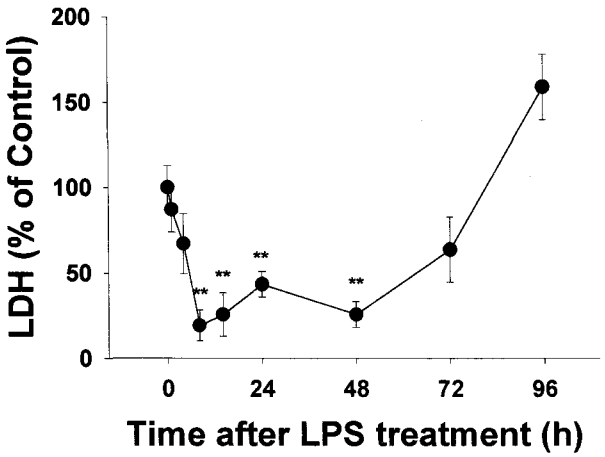

Fig. 3. Effects of LPS on lactate dehydrogenase (LDH) release in heart perfusates after ischemia/reperfusion insult (n=8-9 rats). Isolated hearts from rats treated with LPS during each time period were equilibrated for 15 min. After ischemia-reperfusion, coronary effluent of each treated group was collected and LDH released was measured. **p<0.01 vs control (LPS 0 h).

Effects of LPS on 6-keto-PGF1α release

In nonischemic control hearts, basal 6-keto-PGF1α release into the coronary effluent amounted to 0.34±0.04 ng/ml at the beginning of the experiment (Table 2). After ischemia, at the start of reperfusion, 6-keto-PGF1α release increased to 0.63±0.07 ng/ml and during the reperfusion, 6-keto-PGF1α was progressively decreased to 0.31±0.04 at 30 min of reperfusion. Hearts treated with LPS for 1, 4, 8, 14, 24, and 96 h showed the similar results as in control hearts. However, treatment with LPS for 48 and 72 h resulted in a significant increase in 6-keto-PGF1α levels and there were no further increase after ischemic insult.

Myocardial PGHS analysis

To determine whether the increase of PGI_2 levels by LPS treatment for 48 and 72 h resulted from PGHS protein induction, Western blot analysis was performed. Fig.4 is an immunoblot of left ventricular myocardial samples which were harvested each time after pretreatments and probed against PGHS-1 and -2. PGHS-1 and PGHS-2 protein induction was increased in hearts treated with LPS for 24, 48, 72, and

Table 2. Effects of LPS on prostacyclin release from heart perfusate

	Prostacyclin (ng/ml)		
	Preischemia	Postischemia	Postreperfusion
LPS 0h	0.34±0.04	0.63±0.0.7††	0.31±0.04
LPS 1h	0.65±0.03*	1.18±0.01*††	0.29±0.01
LPS 4h	0.58±0.09*	0.96±0.14†	0.09±0.04**††
LPS 8h	0.36±0.05	0.57±0.08†	0.22±0.04
LPS 14h	0.27±0.02	0.45±0.05†	0.30±0.02
LPS 24h	0.40±0.03	0.63±0.03†	0.30±0.07
LPS 48h	1.28±0.23**	1.28±0.13**	0.66±0.06*†
LPS 72h	1.23±0.20**	1.23±0.14**	0.93±0.23*†
LPS 96h	0.24±0.01	0.47±0.05	0.25±0.27

Isolated hearts from rats treated with LPS during each time period were perfused with Krebs Hensleit buffer for 15 min (preischemia). After 25 min global ischemia (postischemia), hearts were reperfused with the same buffer for 30 min (postreperfusion).

*p<0.05, **p<0.01 vs control (LPS 9hr)

†<0.05, ††p<0.01 vs preischemia of each group

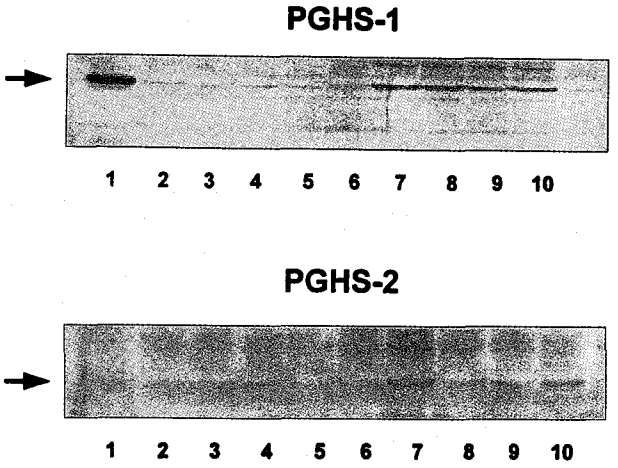

Fig. 4. Prostaglandin H synthase (PGHS)-1 and PGHS-2 protein expression in rat heart samples by western blot analysis. Lane 1, RSV; Lane 2, LPS 0 h; Lane 3, LPS 1 h; Lane 4, LPS 4 h; Lane 5, LPS 8 h; Lane 6, LPS 14 h; Lane 7, LPS 24 h; Lane 8, LPS 48 h; Lane 9, LPS LPS 72 h; Lane 10, LPS 96 h.

96 h. However, no such PGHS-2 band was detected from either vehicle-treated hearts or hearts at other time points after LPS treatment.

Effects of indomethacin on LPS-induced cardioprotection

In order to investigate whether the cardioprotective effects of LPS resulted from PGI_2, indomethacin was applied as an inhibitor of PGI_2 production. For in vitro treatment of indomethacin, rats were pretreated with LPS 4 h or 48 h before, and then indomethacin (1 μmole/l) was added in perfusion solution 30 min prior to ischemic insult (Fig. 5). In control hearts, indomethacin significantly increased LVDP (58.0±2.1 mmHg), +dP/dt (156.27±13.5 mmHg/sec), and DP (15.57±0.81) after I/R compared to those of vehicle treated hearts (18.5±5.8 mmHg, 55.63±24.4 mmHg/sec, 9.3±0.5, respectively). Although it was

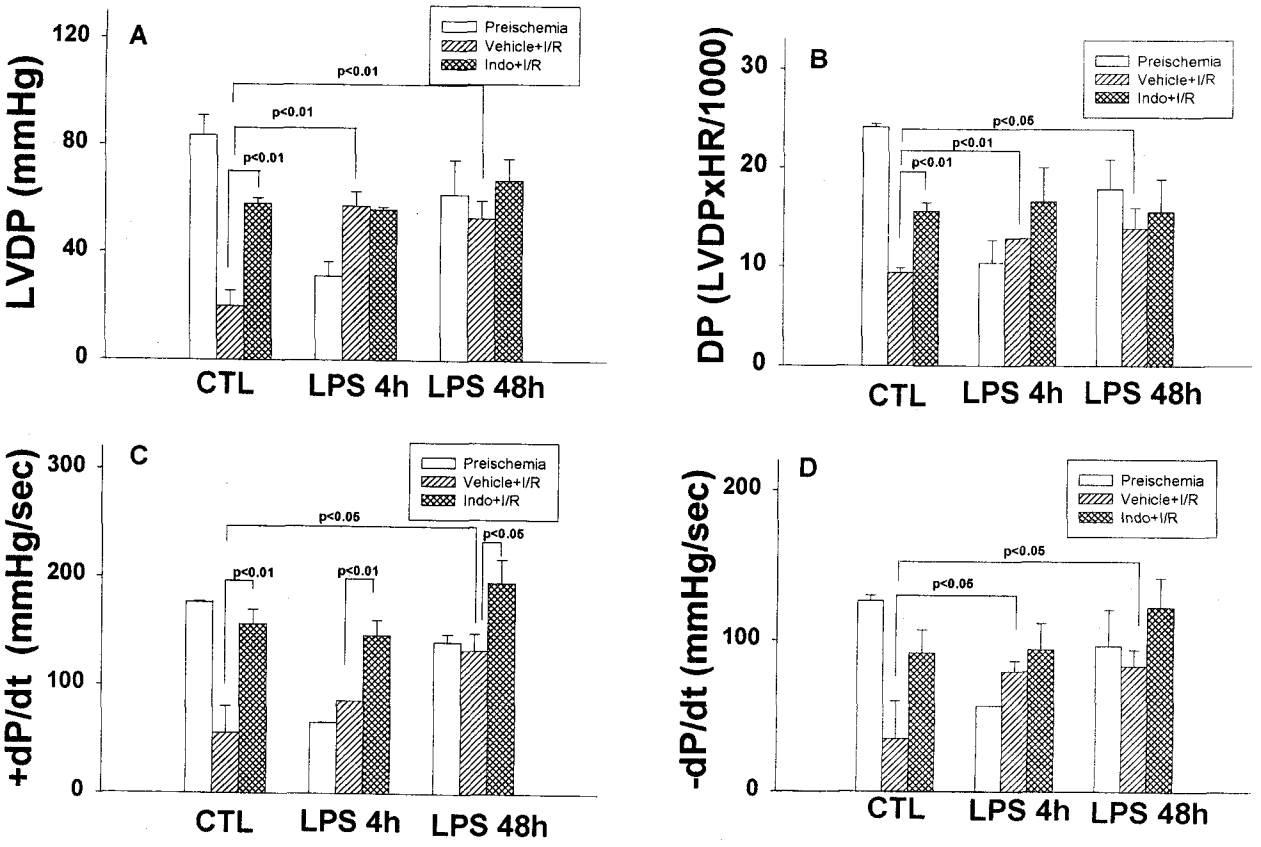

Fig. 5. Effects of indomethacin in vitro treatment on LPS-induced cardioprotection. Rats were pretreated with LPS for 4 or 48 h before and then indomethacin (1 μmole/l) was added in perfusion solution 30 min prior to ischemic insult. Before and after 25 min-ischemia/ 30 min-reperfusion (I/R), hemodynamic parameters were determined (n=5-6 rats). A, left ventricular developed pressure (LVDP); B, double product (DP=LVDP x heart rate /1000); C, +dP/dt; D, -dP/dt.

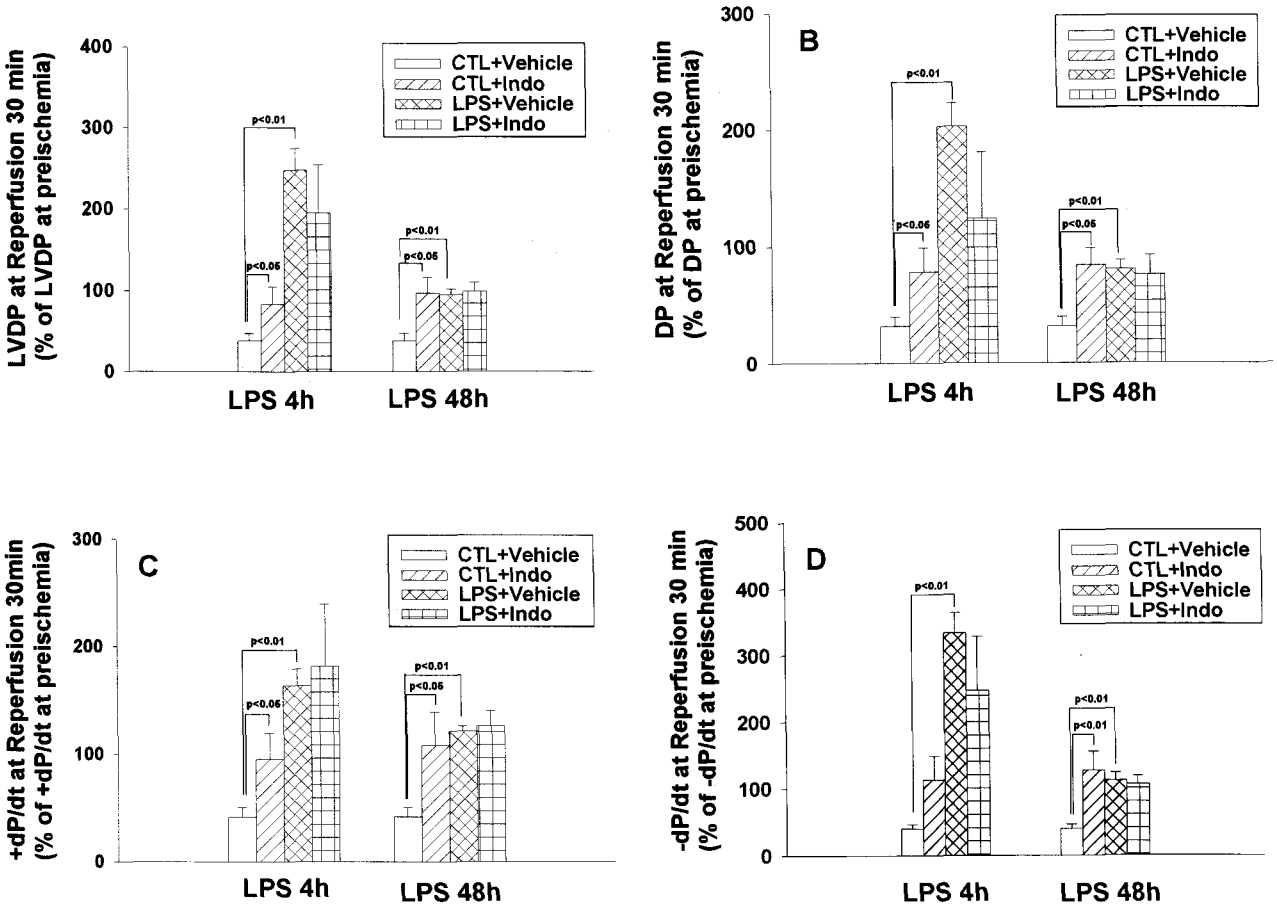

Fig 6. Effects of indomethacin in vivo treatment on LPS-induced cardioprotection. Indomethacin of 2 mg/kg in 10 μM Na_2CO_3 was used in intravenous injection. In hearts treated with LPS for 48 h, indomethacin was treated once a day for two days and hearts were excised 30 min after the final treatment. In hearts treated with LPS for 4 h, indomethacin was injected 30 min before isolation of heart (n=5-6). Before and after 25 min-ischemia/ 30 min-reperfusion (I/R), hemodynamic parameters were determined. Data were expressed as a percentage of the level of each parameter at preischemic state, which meaned cardiac recovery of each parameter against I/R injury (n=8-9 rats). A, left ventricular developed pressure (LVDP); B, double product (DP=LVDP x heart rate /1000); C, +dP/dt; D, -dP/dt.

not statistically significant, -dP/dt after I/R was increased by the treatment of indomethacin. In hearts treated with LPS and vehicle, LVDP, +dP/dt, -dP/dt, DP after I/R increased more than those of control hearts. Exceptionally, +dP/dt in LPS 4 h treated hearts with vehicle was relatively less than in control hearts treated with vehicle. Indomethacin increased +dP/dt in hearts treated LPS for 4 or 48 h more than vehicle treated hearts (145.7±14.4 vs 85.5 mmHg /sec or 195.0±22.1 vs 132.7±15.5 mmHg/sec, respectively). Other hemodynamic parameters, including HR, CFR and time to contracture after ischemic insult, did not show any significant difference between vehicle and indomethacin treatment. For in vivo treatment of indomethacin, the concentration of 2 mg/kg in 10 uM Na_2CO_3 was used in intravenous injection. In hearts treated with LPS for 48 h, indomethacin was treated once a day for two days, and hearts were excised 30 min after the final treatment (Fig. 6). In hearts treated with LPS for 4 h, indomethacin was injected 30 min before the isolation of hearts. In hearts treated with saline 4 h or 48 h before, indomethacin increased LVDP, +dP/dt, -dP/dt and DP more than in vehicle treated hearts. In hearts treated with LPS 4 h or 48 h before, however, indomethacin did not show any significant effects. Other hemodynamic parameters HR, CFR, and time to contracture after ischemic insult did not

show any significant change by vehicle or indomethacin treatment.

Effects of indomethacin on 6-keto-PGF1a release

In vitro treatment of vehicle inhibited the increase of 6-keto-PGF1$_\alpha$ release after ischemic insult (Table 3). Especially, 6-keto-PGF1$_\alpha$ release after ischemic insult was completely blocked by the indomethacin treatment in vitro. When indomethacin was treated in vivo, basal increase of 6-keto-PGF1$_\alpha$ by LPS

Table 3. Effects of indomethacin in vitro treatment on prostacyclin release in control or LPS-treated hearts

	Prostacyclin (ng/ml)		
	Preischemia	Postischemea	Postreperfusion
Control (CTL)	0.52±0.08	0.93±0.28*	0.61±0.13
CTL+Vehicle	0.36±0.12	0.43±0.07*	0.60±0.23
CTL+Indomethacin	0.36±0.09	0.10±0.09**†	0.05±0.02**††
LPS 4h	0.69±0.12	1.28±0.31†	0.38±0.10
L4+Vehicle	0.35±0.19	0.31±0.08**	0.26±0.13
L4+Indomethacin	0.60±0.12	0.08±0.03**††	0.10±0.05*††
LPS 48h	1.16±0.10	1.18±0.03	0.06±0.02††
L48+Vehicle	0.54±0.12**	0.79±0.19	0.18±0.04
L48+Indomethacin	0.53±0.10**	0.05±0.01**††	0.25±0.08

Isolated hearts from rats treated with LPS during each time period were perfused with Krebs Hensleit buffer for 15 min (preischemia) and perfused again for 30 min with the same buffer containing vehcle or indomethacin. After 25 min global ischemia (postischemia). hearts were reperfused with Krebs Hensleit buffer without behicle or indomethacin for 30 min (postreperfusion).
*$p<0.05$, **$p<0.01$ vs vehicle or indomethacin untreated hearts of each group
$^{\dagger}<0.05$, $^{\dagger\dagger}p<0.01$ vs preischemia of each group

Table 4. Effects of indomethacin in vivo treatment on prostacyclin release in control or LPS-treated hearts

	Prostacyclin (ng/ml)		
	Preischemia	Postischemea	Postreperfusion
Control (CTL)	0.36±0.12	2.56±0.19††	0.81±0.19
CTL/Vehicle	0.24±0.05	1.21±0.20**††	0.61±0.05†
CTL/Indomethacin	0.30±0.06	1.18±0.44	0.32±0.04*
LPS 4hr (L4)	0.69±0.12	1.28±0.31	0.38±0.10
L4/Vehicle	0.65±0.07	1.87±0.32††	0.19±0.03†
L4/Indomethacin	0.48±0.07	1.87±0.32††	0.19±0.03†
LPS 48hr (L48)	1.16±0.10	1.18±0.03	0.06±0.02††
L48/Vehicle	0.41±0.13**	0.98±0.29	0.42±0.07**
L48/Indomethacin	0.36±0.13**	1.08±0.39††	0.25±0.08

Isolated hearts from rats treated with LPS and/or indomethacin during each time period were perfused with Krebs Hensleit buffer for 15 min (preischemia). After 25 min global ischemia (postischemia), hearts were reperfused with the same buffer for 30 min (postreperfusion).
*$p<0.05$, **$p<0.01$ vs vehicle or indomethacin untreated hearts of each group
$^{\dagger}<0.05$, $^{\dagger\dagger}p<0.01$ vs preischemia of each group

pretreatment for 48 h or 72 h was disappeared (Table 4). About 2-3 fold increase in 6-keto-PGF1$_\alpha$ release after ischemic insult was not attenuated by indomethacin in vivo treatment.

DISCUSSION

Endotoxin administration to guinea pigs resulted in a progressively increasing myocardial dysfunction and altered inotropic responsiveness of heart muscles isolated from in vivo extracardiac influences of the shock state (Parker & Adams, 1985). In our study, the endotoxin-induced myocardial depression was maximal at 4-8 h. Therefore, it occured at an earlier time after endotoxin treatment than previous reports by other investigators using different methods to evaluate cardiac function. The cause of LPS-induced cardiac dysfunction can be explained in several ways. Priano et al. (1971) reported that cardiac output remained low for up to 24 h after endotoxin administration (5 mg/kg) to unanesthetized dogs. However, they attributed the low cardiac output to deficient venous return and ventricular filling and did not consider myocardial depression as a contributing factor. Hinshaw et al (1972, 1973, 1974a, 1974b, 1979), in a series of studies, reported evidence of heart failure in working canine hearts 5 h~7 h (Hinshaw et al, 1973), 4 h~6 h (Hinshaw et al, 1974a, 1974b; Archer et al, 1975), and 6 h~9 h (Hinshaw, 1972, 1979) after treatment with lethal doses of endotoxin. It has been reported that sublethal doses of LPS can increase myocardial tolerance to a subsequent challenge with ischemia and reperfusion (Brown et al, 1989; Song et al, 1994). Many reports suggested that LPS-induced cardioprotection may be associated with delayed protection. This delayed protection differs from "classical" ischemic preconditioning, which is immediately present but wanes within 1~2 h (Murry et al, 1986). Endotoxemia induces protective states in other organ systems (Frank et al, 1980), including a delayed phase of myocardial adaptation to ischemia. Endotoxemia 24 h~36 h prior to a global ischemic insult improves left ventricular function recovery (Brown et al, 1989a, Bensard et al, 1990). Mediators of endotoxin effects, especially interleukin-1 and tumor necrosis factor (Cybulsky et al, 1988) given 36 h prior to an ischemic insult, also demonstrate cardioprotective effects in the isolated perfused hearts (Brown et al, 1992). Furthermore, the use of the detoxified endotoxin derivative monophosphoryl lipid-A (MPL) demonstrated ischemic cardioprotection 24 h after treatment (Nelson et al, 1991). As a result, the treatment of LPS or its derivatives has resulted in enhanced myocardial tolerance to ischemia and reperfusion many hours after their administration in the isolated heart. However, the precise nature of myocardial protection remains unknown. In this study, we tried to delineate the temporal appearance of LPS-induced cardioprotection. Our result demonstrated temporal biphasic cardioprotection of LPS, one of early protection which shows low basal contractility but rapid induction of cardioprotection (4 h ~14 h) against ischemia/reperfusion injury and the other delayed protection which shows normal basal contractility (24 h~72 h, partially at 96 h). LPS-induced early protection has not been reported by other investigators. Brown et al (1989a) proposed that hearts isolated from rats pretreated with endotoxin 24 h before isolation, not 1 h before isolation, showed an increased myocardial function (decreased injury) after ischemia and reperfusion. Yao et al (1993) demonstrated that pretreatment with MPL 24 h before ischemia produced a significant reduction in myocardial infarct size, but not in heart pretreated with MPL for 1 h. As shown in the two studies, they worked with hearts which had only two injection periods, although they concluded that LPS induced delayed cardioprotection. We also could not find any protective effect in hearts treated with LPS 1 h before, and LPS induced cardioprotection was observed 4 h before LPS treatment. Recently, Song et al (1994) have shown that Escherichia coli endotoxin administration to rats 8 h, but not 1 h, before coronary artery occlusion in vitro was markedly antiarrhythmic. The development of enhanced tolerance to ischemia/reperfusion may be related to the augmentation of intracellular antioxidant defences since endotoxin has been shown to increase catalase activity in rat myocardium (Brown et al, 1989a), superoxide dismutase (SOD) activity in rat lung (Frank et al, 1980), and mitochondrial manganese- dependent SOD (MnSOD) activity in rat liver (Dougall & Nick, 1991). In addition to the changes in intracellular antioxidant system, the elevation of heat shock proteins including the inducible 72 kDa HSP (HSP70i) (Currei et al, 1988, 1993; Marber et al, 1993; Hutter et al, 1994) can be a candidate of endotoxin induced myocardial defences. However, controversies still remain. It seems that low-dose LPS may exert several

biological effects and that the protection observed against ischemia-reperfusion injury in vivo may not be ascribed completely, if at all, to the increased endogenous antioxidant activity or the increased induction of HSP70i.

PGI_2 is the major prostaglandin produced in hearts. There are several evidences that suggest the role of PGI_2 as a cardioprotective mediator ((Lefer et al, 1978; Ogletree et al, 1979; Jugdutt et al, 1981; Schrir et al, 1982; Nelin & Becker, 1983), while other evidences suggest PGI_2 as a reperfusion-induced ventricular failure factor (Karmazyn, 1986). PGI_2 is capable of inhibiting lysosomal enzyme release (Marone et al, 1980) and the generation of oxygen free radicals from stimulated neutrophils (Fantone & Kinnes, 1983). Furthermore, other studies have suggested that iloprost, a stable analogue of PGI_2, might reduce generation of oxygen radicals at reperfusion (Thiemermman et al, 1984), probably by interfering with neutrophil function (Simpson et al, 1987). Thus, we postulated that LPS might stimulate PGI_2 release in hearts, like in other various cells or tissues and that PGI_2 might be another candidate which mediated LPS-induced cardioprotection. At the begining of reperfusion, just after ischemia, PGI_2 release was increased approximately by two fold. This was in accordance with the findings that PGI_2 levels considerably increased at the very beginning of reperfusion (Woditsch & Karsten, 1992). Myocardial ischemia is known to be associated with enhanced lipid degradation, arachidonic acid accumulation, and prostaglandin release during reperfusion (Engels et al, 1990). This will result in some initial overflow of PGI_2 into the coronary effluent at the beginning of reperfusion as was also seen in this study. Woditsch et al (1992) said that the elevation of PGI_2 might be the cause of the protection or myocardial cell integrity. This was evidenced by the deleterous effects of indomethacin on myocardial function. Vegh et al (1990) reported that the protective effect of preconditioning, induced by repeated short ischemic insult, was lost in the presence of the cyclooxygenase inhibitor sodium meclofenamate (2 mg/kg). This report suggested that PGI_2, especially the increased level of PGI_2 in hearts treated with LPS for 48 h and 72 h, might act as an endogenous factor which mediate the protective effect of LPS. In hearts treated with LPS for 48 h or 72h, basal PGI_2 release significantly increased and maintained until the end of ischemic insult. In our Western blot results of PGHS, which is the rate limiting enzyme of PGI_2 production from arachidonic acid, LPS induced the protein expression not only of PGHS-1, a constitutive isozyme of PGHS, but also of PGHS-2, an inducible isozyme, at 24 h after treatment and maintained until 96 h. Therefore, the increase of basal PGI_2 released in hearts treated LPS for 48 and 72 h might be resulted from the increased induction of PGHS, but we could not exclude any other enzymatic steps such as phospholipase A_2. Despite the increase in PGHS, it was not clear why LPS-treated hearts 24 or 96 h prior to isolation did not show the elevation of basal PGI_2 production. In this study, although the augmentation of PGI_2 release in the coronary effluent at the beginning of reperfusion was inhibited by indomethacin treatment in vitro, we could not find any deleterious effects of indomethacin on LPS-induced cardioprotection. In addition, there were no significant changes in LPS-induced cardioprotection by indomethacin treatment in vivo, although indomethacin inhibited the increase of basal PGI_2 release. Thus, PGI_2 might not be a mediator in LPS-induced preconditioning. On the contrary, indomethacin recovered left ventricular deterioration after ischemia /reperfusion by in vivo and in vitro treatment, especially in control hearts. Experimental evidence from the use of nonsteroidal anti-inflammatory drugs (NSAIDs) has produced conflicting results with respect to their influence on the response of the heart to ischemia produced by coronary ligation (Ogletree & Lefer, 1976; Halushka et al, 1977; Capurro et al, 1979). The administration of large concentrations of PGs, including PGI_2, has developed beneficial effects, such as antiarrhythmic influence as well as protection of the ischemic heart (Karmazyn & Dhalla, 1983). However, with respect to arrhythmias, low concentrations of PGs have been shown to produce direct arrhythmogenic effects (Moffaat et al, 1986, 1987, 1989). These suggest that the concentration of PGI_2 was important in the level of its cardioprotective effects. In our experiment, it seems that prostaglandin did not act as a failure factor at least in this experimental model in LPS-treated hearts, considering indomethacin did not show any significant effect on LPS-induced cardioprotection.

Taken together, our results suggest that LPS-induced cardioprotection against ischemia/reperfusion injury appeared 4 h after treatment and maintained until 96 h. However, this effect of LPS might not be meadiated by PGI_2 release.

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