British Journal of Pharmacology (1997) 120, 305–311

Reperfusion injury in the endotoxin-treated rat heart: reevaluation of the role of nitric oxide

B.C. Yang, L.Y. Chen, *T.G.P. Saldeen & [1]J.L. Mehta

Department of Medicine, University of Florida College of Medicine and the V.A. Medical Center, Gainesville, FL, U.S.A. and *Department of Forensic Medicine, University of Uppsala, Sweden

1 The role of nitric oxide (NO) in ischaemia-reperfusion injury to the heart continues to be debated.

2 The role of NO released during endotoxemia on myocardial reperfusion injury was examined in rats given saline or lipopolysaccharide (LPS, 10 mg kg^{-1}).

3 Aortic rings from LPS-treated rats showed a markedly decreased contractile response to both noradrenaline (NA) and U46619, and a diminished relaxation response to acetylcholine, thrombin and aggregating platelets. Treatment of rat aortic rings from LPS-treated rats with the NO synthesis inhibitor N^{ω}-nitro-L-arginine (L-NOARG) reversed the diminished contractile response to NE and U46619.

4 Before ischaemia-reperfusion, baseline force of cardiac contraction (FCC) and coronary perfusion pressure (CPP) were lower and coronary flow was higher in hearts from LPS-treated rats (all $P<0.05$ vs. saline-treated group). Treatment of hearts from LPS-treated rats with L-NOARG increased baseline FCC and CPP.

5 After ischaemia-reperfusion, hearts from saline-treated rats showed a $36\pm5\%$ fall in FCC, a $38\pm6\%$ rise in CPP and a $38\pm5\%$ fall in coronary flow, whereas hearts from LPS-treated rats revealed only a $16\pm9\%$ fall in FCC, a $10\pm3\%$ rise in CPP and a $20\pm4\%$ fall in coronary flow (all $P<0.05$ vs. changes in saline-treated group). Fewer hearts from LPS-treated rats developed reperfusion arrhythmias (6% vs. 60% hearts from saline-treated rats, $P<0.02$). Myocardial superoxide dismutase activity was higher in the LPS-treated group ($P<0.05$).

6 NO synthesis, measured as formation of nitrite, was higher ($P<0.05$) in cardiac and aortic tissues from LPS-treated rats. Prostacyclin (PGI_2) release in coronary effluent was greater in LPS-treated rat hearts ($P<0.05$ vs. saline-treated rats).

7 Thus LPS-treated hearts demonstrate a basal decrease in FCC and coronary vascular resistance. These hearts demonstrate a modest protection from reperfusion injury. Induction of NO synthesis, and possibly PGI_2 release, may underlie cardioprotection from ischaemia-reperfusion.

Keywords: Ischaemia-reperfusion; lipopolysaccharide; nitric oxide

Introduction

The basal release of small amounts of nitric oxide (NO) by a constitutive, Ca^{2+}-dependent NO synthase in vascular endothelium plays an important role in the control of blood pressure and regional blood flow by stimulating the accumulation of guanosine 3′:5′-cyclic monophosphate (cyclicGMP) in vascular smooth muscle cells (Furchgott & Vanhoutte, 1989; Moncada *et al*., 1991). Reperfusion of previously ischaemic myocardial regions results in endothelial dysfunction, increase in coronary vascular resistance, and extension of myocardial injury (Mehta *et al*., 1989; McLenachan *et al*., 1990; Tsao *et al*., 1990; Yang *et al*., 1993; Chen *et al*., 1995). Much of this phenomenon of 'reperfusion injury' has been attributed to inactivation or loss of release of NO and release of superoxide anions (Tsao *et al*., 1990; Chen *et al*., 1995). Therefore, augmentation of synthesis, release or activity of NO during ischaemia-reperfusion should be beneficial (Salvemini & Mollace, 1994). However, the effects of NO on myocardial ischaemia/reperfusion injury have been controversial (Feigl, 1988; Beckman *et al*., 1990; Johnson *et al*., 1990; Matheis *et al*., 1992; Siegfried *et al*., 1992; Weyrich *et al*., 1992; Patel *et al*., 1993; Yang *et al*., 1993). Lefer's group demonstrated that administration of NO precursor L-arginine (Weyrich *et al*., 1992) or NO donors, acidified sodium nitrite (Johnson *et al*., 1990) or morpholino-sydnonimine 1 (SIN-1) (Siegfried *et al*., 1992), mitigates against ischaemia-reperfusion-induced myocardial injury and endothelial dysfunction in anaesthetized cats. The salutary effects of L-arginine against myocardial ischaemia-reperfusion injury have also been shown in rat isolated buffer-perfused hearts (Yang *et al*., 1993). On the other hand, Beckman *et al*. (1990) have proposed that formation of peroxynitrite from superoxide anion and NO may be an important determinant of tissue injury following ischaemia-reperfusion. Matheis *et al*. (1992) showed that administration of an NO synthesis inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) protected against myocardial reoxygenation injury in pigs subjected to cardiopulmonary bypass. Patel *et al*. (1993) also showed that administration of L-NAME reduced infarct size following sustained coronary artery occlusion and reperfusion in rabbit hearts *in situ*. The protective effects of L-NAME against ischaemia have also been demonstrated in a rat cerebral ischaemia model by Buisson *et al*. (1992).

There are two different isoforms of NO synthase, first the constitutive isoform which catalyses formation of small amount of NO, the second inducible form which results in release of large amounts of NO and plays an important role in defending host against invading microorganisms (Furchgott & Vanhoutte, 1989; Moncada *et al*., 1991). Cytotoxic effects of a large amount of NO have been shown in tumour cells, hepatocytes, and neural cells (Billiar *et al*., 1981; Stuehr & Nathan, 1989; Dawson *et al*., 1992). NO release also has been shown to mediate negative inotropic effects of cytokines (Finkel *et al*., 1992; Balligand *et al*., 1993) and of bacterial products, such as lipopolysaccharide (LPS) (Brady *et al*., 1992). It may be speculated that release of large amounts of NO either directly or by formation of large amounts of peroxynitrite, may be detrimental to myocardial tissues,

[1]Author for correspondence at: Box 100277, University of Florida, Gainesville, FL 32610, U.S.A.

particularly in states of ischaemia-reperfusion when tissues are at risk of free-radical-mediated injury (Thompson & Hess, 1986). The present study, therefore, employed administration of LPS to rats to investigate the role of elevated NO level on myocardial ischaemia-reperfusion injury and vascular reactivity in a rat model.

Methods

These studies were approved by the University of Florida animal research committee, and all local, state and federal guidelines were followed in the conduct of the study.

Treatment of rats

Male Sprague-Dawley rats weighing 275–300 g (heart weight 900–1000 mg) were injected intraperitoneally with: (a) 0.55–0.6 ml of saline ($n=19$), (b) LPS (from *Escherichia coli*, serotype 0127:BB) 10 mg kg^{-1} in saline ($n=20$).

Isolated perfused hearts

Six hours after the treatment, rats were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}) intraperitoneally. Blood was drawn from the common carotid artery into 3.8% sodium citrate (v:v = 9 : 1) to harvest platelets. The hearts and aortae were rapidly excised and placed in ice-cold Krebs-Henseleit (K-H) buffer composed of (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11. Within 1 min, the hearts were transferred to a perfusion apparatus and perfused via the aortas with oxygen-saturated (95% O_2 + 5% CO_2) buffer solution kept at 37°C, pH 7.4, at constant perfusion flow (5.5–6.0) by a MasterFlex pump (model 7016-21, Cole-Palmer Instrument Co., Chicago, IL) or constant perfusion pressure (80 cm H_2O), according to the modified Langendorff procedure (retrograde, no-recirculation) (Neely & Rovetto, 1975; Yang *et al*., 1993). The heart was placed in a semi-closed, circulating water warmed (37°C) air chamber, paced at 300 beats min^{-1} with an atrial placing Medtronic 5320 (Medtronic, Inc., Minneapolis, MN) pacemaker. Coronary perfusion pressure (CPP, in constant flow hearts) was measured via a catheter placed just proximal to the aorta and connected to a Gould Statham P23ID pressure transducer (Hato Ray, Puerto Rico). To ascertain the changes in coronary resistance, some hearts were perfused at constant pressure. In constant pressure perfused hearts, coronary flow was also measured. To measure the force of cardiac contraction (FCC), the aorta was fixed at 2–3 mm above the root, a metal hook was connected to the apex of the heart, and connected to a force transducer (Kristler Morse, Redmond, WA) via a lever with the fulcrum at the middle, as described previously (Yang *et al*., 1993; 1994; Yang & Mehta, 1994a). This technique measures FCC across the long-axis, and reflects changes in contractility in the intact heart. The myocardial preload during equilibration was kept at about 2 g. Both the CPP and FCC were continuously recorded on a four-channel recorder (Astro-Med, West Warwick, RI). Heart rate was monitored from the fast speed tracing of the cardiac contraction signals.

Preparation of aortic rings

Rat thoracic aorta was placed in ice-cold K-H buffer, cleaned of fat and loose connective tissues and cut into 4 to 5 mm rings. The rings were mounted onto wire stirrups, connected to Grass force-displacement transducers FT03 (Grass Instrument, Quincy, MA) and suspended in custom-designed tissue-organ baths filled with oxygen-saturated (95% O_2 + 5% CO_2) Krebs-Ringer buffer (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 12.5, Na-EDTA 0.01 and glucose 11.1, pH 7.4). The rings were then stretched to and maintained at a preload of 5 g, and allowed to equilibrate for about 2 h. During equilibration, the buffer was replaced every 30 min and continuously aerated with 95% O_2 + 5% CO_2, as described previously (Yang & Mehta, 1994b).

Preparation of platelets

Anticoagulated blood was centrifuged at 100 *g* for 10 min at room temperature to obtain platelet-rich plasma (PRP). An aliquot of PRP (final platelet count 5×10^8 cells ml^{-1}) was saved for aggregation in response to ADP (Yang & Mehta, 1994b). The remaining PRP from saline-treated rats was centrifuged at 1000 *g* for 20 min at 4°C. The pellet was washed twice with Tris-sodium-glucose (TSG) buffer (composition in mM: Tris-HCl 15, NaCl 134, EDTA 1 and glucose 5, pH 7.4) and suspended in a final platelet concentration of 10^9 cells min^{-1} in TSG buffer. These washed platelets showed a normal aggregatory response to thrombin (1 u ml^{-1}) (Yang & Mehta, 1994b). Platelets were separated from PRP about 30 min before being suspended in the organ baths. During this period, washed platelets were kept on ice. These platelets were used to examine vasorelaxation.

Experimental protocols

Ischaemia-reperfusion Four hearts from saline-treated rats were continuously perfused with K-H buffer at constant flow (5.5–6.0 ml min^{-1}) for 90 min and served as sham controls. Other hearts (10 from saline-treated rats and 10 from LPS-treated rats) were equilibrated for 20 min under continuous flow, then subjected to 40 min of total ischaemia (stop perfusion) followed by 30 min of reperfusion. FCC and CPP were continuously recorded. Coronary effluent was collected several times during the experiment and stored at −70°C for measuring 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), the stable metabolite of PGI_2. After the experiment, hearts were sealed and stored at −70°C for measuring myocardial creatine kinase (CK), superoxide dismutase (SOD) and malondialdehyde (MDA).

Five hearts from saline-treated rats and 6 hearts from LPS-treated rats were perfused at constant pressure (80 cmH_2O), stabilized for 20 min, then subjected to 40 min of ischaemia and 30 min of reperfusion. FCC and coronary flow were continuously recorded. Four hearts from LPS-treated rats were perfused with K-H buffer containing the NO synthesis inhibitor N^{ω}-nitro-L-arginine (N^{ω}[nitroamidino]-L-2,5-diaminopentanoic acid, L-NOARG, 500 μM) during equilibration, perfused at constant flow and then subjected to 40 min of ischaemia and 30 min of reperfusion.

Aortic ring reactivity Parallel sets of aortic rings were exposed to cumulative concentrations of noradrenaline free base (NA, 0.1 nM–10 μM) or the thromboxane (TX) A_2 analogue U46619 (10 fM–1 nM) to determine the vascular contractile response. Some rings from LPS-treated rats were incubated with L-NOARG (500 μM) for 5–10 min, and then exposed to NA or U46619. Other parallel sets of aortic rings were contracted with NA to obtain ≈2–3 g of tone (≈70–80% of maximal tone), then exposed to acetylcholine chloride (ACh, 0.1 nM–10 μM), thrombin (0.1–1.0 u ml^{-1}) or washed autologous platelets (10^6–10^8 cells ml^{-1}) so as to determine the vasorelaxant response. Since platelets cause relaxation of arteries with intact endothelium and cause contraction of arteries without functional intact endothelium (Yang & Mehta, 1994c), platelets were suspended in the organ bath to not only test endothelium-dependent vasorelaxation, but also to examine endothelial integrity. After each experiment, aortic rings were weighed for calculation of vasoconstriction in g (tone) mg^{-1} (tissue).

Determination of NO synthase activity in myocardial and aortic tissues

NO synthase activity was determined by measuring nitrite formation in myocardial homogenates or aortic tissues. In

brief, hearts and aortae from saline-treated and LPS-treated rats were washed with K-H buffer. Hearts were homogenized in Ca^{2+}-rich buffer, and the homogenate and aortic segments were then incubated with L-arginine (5 mM) for 1 h. Aortic segments were then homogenized. The homogenates were centrifuged at 3000 *g* for 30 min at 4°C. Protein in the myocardial supernatant was measured according to the method of Lowry *et al*. (1951). Nitrite in the myocardial and aortic supernatants was measured by the Griess reaction (Green *et al*., 1982). Nitrite in myocardial supernatant was expressed as nmol mg^{-1} protein. Nitrite in aortic supernatant was expressed as nmol mm^{-1} length of the vessel.

Myocardial SOD and MDA measurements

Myocardium was homogenized in 5% distilled water. Aliquots of homogenate were centrifuged at 5000 *g* for 60 min at 4°C. The superoxide dismutase (SOD) activity in the supernatant was measured by spectrophotometric monitoring of the SOD inhibitable autooxidation of pyrogallol as described by Marklund and Marklund (1974). One unit of SOD was defined as inhibition of autooxidation of pyrogallol by 50%. Malondialdehyde (MDA) content of the myocardial homogenate was measured by thiobarbituric acid method (Ohkawa *et al*., 1979). Protein in the myocardial supernatant was measured according to the method of Lowry *et al*. (1951).

6-keto-$PGF_{1\alpha}$ assay

Prostacyclin release was measured by quantitation of its stable hydrolysis product 6-keto-$PGF_{1\alpha}$ in the coronary effluent by enzyme-linked immunosorbent assay (ELISA) (Folger *et al*., 1991).

Reagents

LPS (derived from *Escherichia coli*, serotype 0127:B8), L-NOARG, NA and other reagents were obtained from Sigma Chemical Co., St. Louis, MO. U46619 ((5Z, 9α,13E, 15S)-11,9-(E.poxymethano) prosta-5, 13-dien-1-oic acid) was a gift from Pharmacia-Upjohn, Kalamazoo, MI. All reagents were fresh made before use.

Statistical analysis

All data are expressed as mean ± s.e.mean. One-way or two-way analysis of variance (ANOVA) followed by Student Newman-Keul's test or Chi-Square was used to evaluate the statistical significance of data. A P value less than 0.05 was considered significant.

Results

Six hours after intraperitoneal administration, rats in the saline group showed no evidence of distress, but rats given LPS appeared lethargic. However, none of the rats died during the 6 h of observation.

Platelet counts and aggregation

In the saline-treated rats, the platelet count in the peripheral blood was $4.5 \pm 1.1 \times 10^9$ cells ml^{-1} and the magnitude of ADP-induced platelet aggregation was 62 ± 3%. In the LPS-treated rats, both platelet count and platelet aggregation were significantly lower ($1.3 \pm 0.71 \times 10^9$ cells ml^{-1} and 30 ± 2%, respectively, both $P < 0.01$ vs. saline-treated rats).

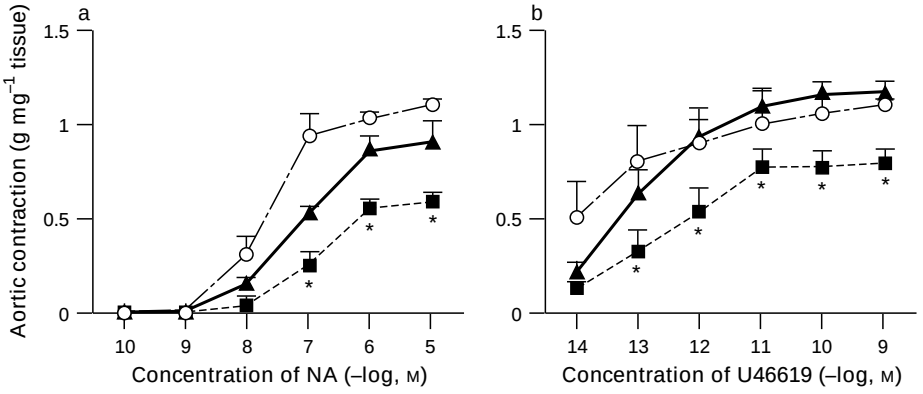

Figure 1 Diminished contractile response of aortic rings from LPS-treated (■) rats to (a) noradrenaline (NA) and (b) U46619 (vs. contractile response of aortic rings from saline-treated (▲) rats). Treatment of aortic rings from LPS-treated rats with the NO synthesis inhibitor L-NOARG (○) restored the contractile response to NA and U46619. Symbols represent data (mean ± s.e.mean) in different groups of rats ($n = 4-10$ in each group). *$P < 0.05$ vs. saline group.

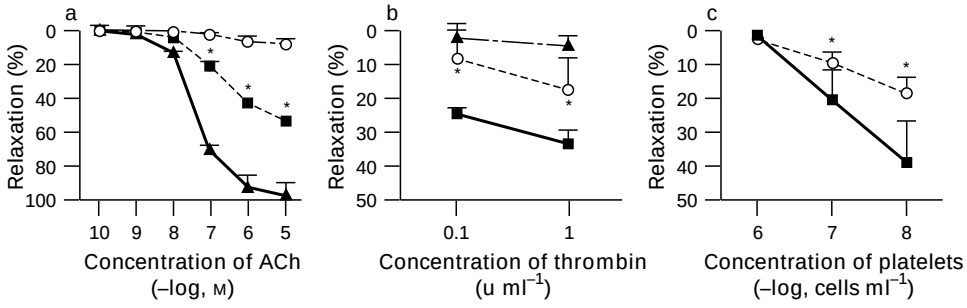

Figure 2 Diminished relaxation of aortic rings from LPS-treated rats to (a) acetylcholine (ACh) (▲ saline; ■ LPS and ○ LPS + L-NOARG), (b) thrombin (■ saline; ○ LPS and ▲ LPS + L-NOARG), and (c) washed platelets (■ saline and ○ LPS) (vs. relaxation of aortic rings from saline-treated rats). Treatment of aortic rings from LPS-treated rats with L-NOARG abolished the relaxation response to ACh and thrombin. Symbols represent data (mean ± s.e.mean) in different groups of rats ($n = 4-10$ in each group). *$P < 0.05$ vs. saline.

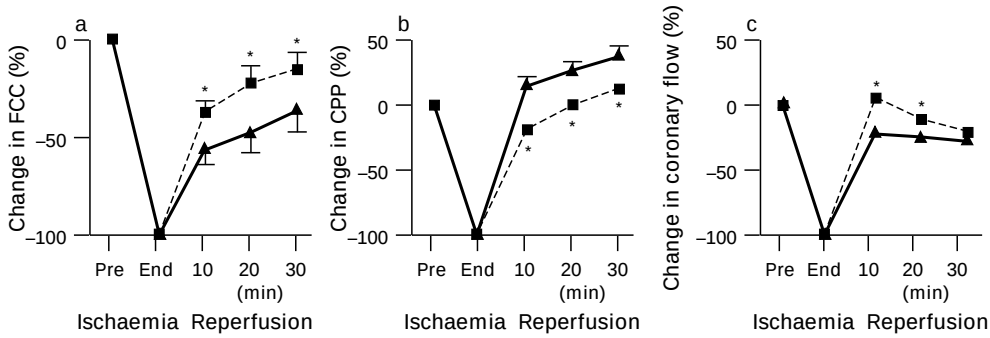

Figure 3 Changes in (a) force of cardiac contraction (FCC), (b) coronary perfusion pressure (CPP) and (c) coronary flow during ischaemia and reperfusion in hearts from rats treated with saline (▲) or lipopolysaccharide (LPS, ■). Hearts from LPS-treated rats show a smaller decrease in FCC and a smaller rise in CPP and preserved coronary flow during reperfusion. Symbols represent data (mean ± s.e.mean) in different groups of rats ($n = 10$ in each group). *$P < 0.05$ vs. control.

Vascular reactivity

As shown in Figure 1, the contraction of aortic rings in response to both NA and U46619 was significantly diminished in the LPS-treated group compared to the saline-treated group ($P < 0.01$). Treatment of aortic rings from LPS-treated rats with L-NOARG restored the contractile response to NA as well as U46619, and the contractile response in the case of NA became somewhat greater than that in saline-treated rats.

The relaxation in response to ACh, thrombin, and platelets was also markedly decreased in rings from LPS-treated rats compared with that in rings from saline-treated rats ($P < 0.01$) (Figure 2). There was no contractile response on addition of platelets. Treatment of rings from LPS-treated rats with L-NOARG abolished the relaxation in response to ACh and thrombin (Figure 2).

Cardiac dynamics during equilibration, ischaemia and reperfusion

In the continuously perfused hearts from saline-treated rats, there were nonsignificant changes in CPP and FCC during a 90 min period of observation.

In the LPS-treated group (constant flow perfused hearts), both CPP and FCC were lower at the beginning of perfusion (CPP: 43 ± 1 vs. 48 ± 1 mmHg; FCC: 6.6 ± 0.4 vs. 9.8 ± 0.7 in the saline-treated group, both $P < 0.05$). Total ischaemia (40 min) followed by reperfusion (30 min) resulted in a marked decrease in FCC and increase in CPP in all hearts (Figure 3). The magnitudes of the decrease in FCC and increase in CPP were less marked in hearts from LPS-treated than those from saline-treated rats ($P < 0.01$) (Figure 3).

In hearts perfused with a constant pressure, coronary flow was higher (8.1 ± 0.5 vs. 7.1 ± 0.5 ml min^{-1}) and FCC was lower (7.0 ± 1.0 vs. 10.0 ± 0.5 g) in the LPS-treated group than in the saline-treated group at the beginning of perfusion (both $P < 0.01$ vs. saline-treated group). Total ischaemia followed by reperfusion resulted in a decrease in FCC and coronary flow in both saline- and LPS-treated groups; however, the magnitude of the decrease was less marked in the LPS-treated group ($P < 0.05$) (Figure 3).

The frequency of transient ventricular fibrillation in hearts from the saline-treated group during reperfusion was 60% (9 of 15 hearts). In contrast, only 6% of the hearts from the LPS-treated group (1 of 16 hearts) showed transient ventricular fibrillation ($P < 0.01$ vs. saline-treated group).

Treatment of hearts from LPS-treated rats with L-NOARG ($n = 4$) resulted in marked increase in FCC (+51%) and CPP (+113%) during 20 min of equilibration. The FCC and CPP did not stabilize for 40 min and these hearts were not subjected to ischaemia-reperfusion.

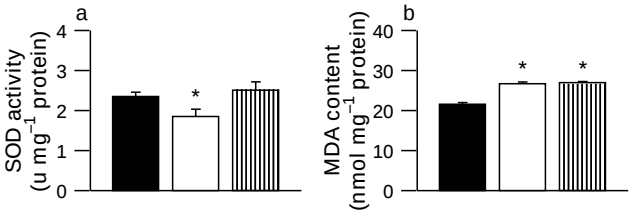

Figure 4 (a) Myocardial superoxide dismutase (SOD) activity was lower in hearts from saline-treated rats (open columns) after ischaemia-reperfusion compared to sham control hearts (solid columns). Hearts from LPS-treated rats (striped columns) showed preservation of myocardial SOD activity. (b) Myocardial malondialdehyde (MDA) content was greater in hearts from saline- or LPS-treated rats compared to sham control hearts. Columns represent mean ± s.e.mean from different groups of rats ($n = 6$ in each group). *$P < 0.05$ vs. sham control.

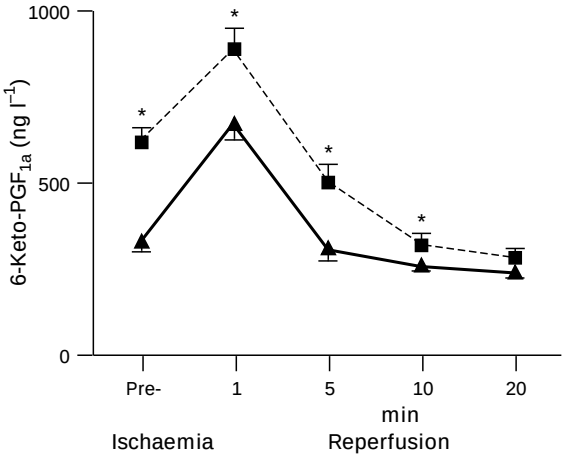

Figure 5 6-Keto-$PGF_{1\alpha}$ (ng l^{-1}) levels in coronary effluents of hearts from saline (▲)- and LPS (■)-treated rats during ischaemia and reperfusion. Symbols represent data (mean ± s.e.mean) in different groups of rats ($n = 5$ in each group). *$P < 0.05$ vs. saline.

Indices of oxidant activity

Myocardial SOD activity was lower in the saline-treated group ($P < 0.05$ vs. sham control hearts) and was preserved in the LPS-treated group after ischaemia/reperfusion. Myocardial MDA content was higher in saline-treated hearts subjected to ischaemia-reperfusion than in sham control hearts ($P < 0.05$). However, treatment with LPS did not affect the ischaemia/reperfusion elevated MDA content of the hearts (NS vs. saline-treated hearts) (Figure 4).

Coronary effluent 6-keto-PGF$_{1\alpha}$

As shown in Figure 5, coronary effluent 6-keto-PGF$_{1\alpha}$ concentrations during the entire experiment were significantly higher in the LPS-treated group than in the saline-treated group ($P<0.01$).

Myocardial and aortic NO synthase activity

Nitrite concentration in the supernates of myocardial homogenates was 3.4 ± 0.1 in the saline-treated group and 4.9 ± 0.1 nmol mg^{-1} protein in the LPS-treated group. The difference between these means was significant ($P<0.05$). Similarly, nitrite concentrations in aortic ring supernates were higher in the LPS-treated rats (mean 2.1 ± 0.3 vs. 1.4 ± 0.2 nmol mm^{-1} segment in saline-treated group, $P<0.05$).

Discussion

In this study, rats treated with LPS showed clear evidence of endotoxaemia, as reflected by a decrease in platelet number and platelet aggregation. In addition, there was suppression of the contractile response of aortic rings to both NA and U46619 and a decrease in the relaxant response to various endothelium-dependent vasodilators. Furthermore, isolated hearts from LPS-treated rats showed diminished coronary vascular resistance and FCC compared to the hearts from saline-treated rats. These changes in the LPS-treated rats were associated with a significant rise in NO synthase activity in myocardial and aortic tissues. LPS administration was also associated with increased PGI_2 release in the coronary effluents. Treatment of hearts and aortae from LPS-treated rats with the NO synthase inhibitor L-NOARG *in vitro* reversed the LPS-induced cardiovascular depression. Taken together, these data indicate that treatment of rats with a single large dose of LPS results in the release of a large amount of NO and haemodynamic alterations.

Schulz *et al.* (1992) have provided conclusive evidence of induction of NO synthase by LPS and cytokines in myocardium. Release of large amounts of NO would be expected to result in systemic hypotension (Schulz *et al.*, 1992; Szalzo *et al.*, 1993). The vascular smooth muscle may become hyporesponsive to several pressor agents, including catecholamines (Wakabayashi *et al.*, 1987; Szalzo *et al.*, 1993). In accordance with these concepts, we found a low coronary vascular resistance and suppressed contractile response of aortic rings from LPS-treated rats to two different stimuli, NA and U46619. The reduced responsiveness of aortic rings to vasoconstrictor stimuli was 'normalized' and coronary vascular resistance in the isolated hearts was increased following pretreatment with the NO synthesis inhibitor L-NOARG. We also found diminished relaxation of aortic rings from LPS-treated rats in response to three different endothelium-dependent vasorelaxants, ACh, thrombin and platelets. Whereas the phenomenon of diminished vasoconstriction can be attributed to release of large amounts of NO in the aortic tissues from LPS-treated rats, diminished endothelium-dependent relaxation may also be due to the inhibitory effect of endotoxin on Ca^{2+}-dependent constitutive NO synthase activity (Parker & Adams, 1993; Graier *et al.*, 1994). Of note is the fact that suspension of platelets caused relaxation of aortic rings from LPS-treated rats albeit less than in saline-treated rats, which indicates that the vascular endothelium after LPS-treatment remained intact.

FCC was markedly reduced in hearts from LPS-treated rats, probably a reflection of the negative inotropic effect of LPS (Brady *et al.*, 1992). Adams *et al.* (1990) found intrinsic myocardial dysfunction during endotoxaemia-independent of changes in coronary flow. In support of a role for LPS-induced NO release in diminished FCC is our observation of 'normalization' of FCC in hearts from LPS-treated rats by L-NOARG treatment.

The effects of NO on myocardial dysfunction induced by ischaemia and reperfusion have been controversial. Whereas early studies suggested a salutary effect of NO on myocardial function following reperfusion injury (Johnson *et al.*, 1990; Siegfried *et al.*, 1992; Weyrich *et al.*, 1992), more recent studies have shown a protective effect of NO inhibitors in the intact animal (Buisson *et al.*, 1992; Matheis *et al.*, 1992; Patel 1993). However, all these studies (Johnson *et al.*, 1990; Buisson *et al.*, 1992; Matheis *et al.*, 1992; Siegfried *et al.*, 1992; Weyrich *et al.*, 1992; Patel *et al.*, 1993) presumed the NO release was altered during ischaemia-reperfusion, and did not differentiate between constitutive and inducible NO release.

The present study was specifically designed to address the issue of LPS and ischaemia-reperfusion-induced myocardial dysfunction. We found that treatment of rats with LPS attenuated the deterioration of myocardial function in response to ischaemia-reperfusion. Reduction in the frequency of transient ventricular arrhythmias during the reperfusion phase in LPS-treated hearts also suggests some cardioprotection.

Protection against reperfusion-mediated myocardial dysfunction by LPS administration in the present study may relate to several mechanisms. Firstly, the release of oxygen free radicals during early stages of reperfusion contributes to myocardial injury (Thompson & Hess, 1986) and endothelial dysfunction (Mehta *et al.*, 1989; McLenachan *et al.*, 1990; Tsao *et al.*, 1990; Yang *et al.*, 1993), mediated at least in part by inactivation of NO. Release of large amounts of NO following LPS administration may have counteracted the detrimental effects of free radicals on the myocardium and coronary vasculature. Secondly, direct inhibition of the generation of superoxide anions by NO (Clancy *et al.*, 1992) may be a mechanism for the cardioprotective effects of LPS. Thirdly, release of PGI_2 in coronary effluent of hearts from LPS-treated group may have contributed to the cardioprotective effects of LPS (Lefer & Arak, 1980). The increase in PGI_2 release could be a direct response to endotoxin administration (Wise *et al.*, 1980). PGI_2 has also been shown to reduce the generation of superoxide anions (Fantone & Kinnes, 1983). Lastly, a decrease in FCC and resistance in the coronary microvasculature in response to NO and PGI_2 may reduce wall stress and oxygen demand and result in a reduction in reperfusion injury.

The ability of LPS to trigger the release of a number of other mediators apart from NO and PGI_2 needs to be recognized, such as cytokines, endothelin, arachidonic acid metabolites, complement factors, bradykinin, histamine, 5-hydroxytryptamine, catecholamines and shock proteins. Many of these mediators could also participate in the determination of cardiac function following reperfusion unrelated to the alterations in NO. In the present study, we only demonstrated the increased release of NO and PGI_2 in the isolated preparation, which may well have been salutary to the heart during ischaemia reperfusion. Since the myocardial ischaemia reperfusion was performed *in vitro*, the LPS-induced release of factors other than NO and PGI_2 should be much less than that *in vivo*. Most of these LPS-released mediators (other than NO and PGI_2) exert cytotoxic and vasoconstrictor effects, and may not be expected to exert beneficial effects during ischaemia-reperfusion. We postulate that the LPS-induced NO and PGI_2 release probably contributes to the observed beneficial effect of LPS-treatment on the isolated heart following ischaemia-reperfusion.

To ascertain the role of NO release, we planned to inhibit the NO synthesis by perfusion of hearts with L-NOARG, but were not able to subject the hearts to ischaemia-reperfusion due to the lack of stabilization of the cardiac preparations. We also examined the effect of *in vivo* administration of L-NAME (an inhibitor of NO synthesis) 30 min before LPS on the LPS-mediated cardiac effect *in vitro* in some rats. In data not presented here, we found a detrimental effect of the administration of combination of L-NAME and LPS on cardiac and systemic haemodynamics resulting in high mortality, similar to data found by Wright *et al.* (1992). In the isolated hearts from

surviving rats, we found no difference in the cardiac performance between the hearts from LPS-treated rats and the hearts from L-NAME + LPS-treated rats. The effect of NO inhibition is thus not clear from these studies; therefore, we did not present these data in the present manuscript. Notably, administration of dexamethasone, which prevents the activity of inducible NO synthase as well as the detrimental effect of other mediators of endotoxic shock, was not found to protect against the haemodynamic effects of LPS (Wright *et al*., 1992). Thus the nature of the specific mediator released in response to LPS administration, which exerts a cardioprotective effect, remains unclear.

In the present study, myocardial SOD activity after ischaemia and reperfusion was higher in LPS-treated rats than in the saline-treated rats, which is consistent with the concept of NO and PGI_2 reducing the generation of superoxide anions (Fantone & Kinnes, 1983; Clancy *et al*., 1992). NO can also combine with superoxide radicals *in vitro* to produce the potentially cytotoxic substance peroxynitrite (Beckman *et al*., 1990). This radical has been shown to oxidize sulphydryl groups and peroxidize membrane lipids (Radi *et al*., 1991). Pearson *et al*. (1991) have indicated that hypoxia enhances the production of an endothelium-derived constricting factor which they propose to be peroxynitrite. Therefore, it is possible that peroxynitrite is produced during ischaemia-reperfusion injury; however, to date the production of peroxynitrite has only been demonstrated *in vitro*. Additionally, due to the short life-time (1.9 s) (Beckman *et al*., 1990) and the high concentration of peroxynitrite required to produce lipid peroxidation (100 μM to 1 mM) (Radi *et al*., 1991), it is unlikely that peroxynitrite plays a significant role in ischaemia-reperfusion-mediated injury.

Conclusion

In summary, administration of LPS results in release of NO and PGI_2. The net effect of these mediators is a decrease in coronary vascular resistance and FCC. The hearts from LPS-treated rats appear to be somewhat protected from the detrimental effects of ischaemia-reperfusion. It is likely that release of large amounts of NO, and possibly PGI_2, is responsible for cardioprotection. However, some other mediator released in response to LPS administration may also exert a cardioprotective effect following ischaemia-reperfusion.

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(Received June 18, 1996
Revised August 20, 1996
Accepted October 2, 1996)