Effects of Endotoxin on Neutrophil-Mediated Ischemia/Reperfusion Injury in the Rat Heart *In Vivo*

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We have previously shown that a nonlethal dose of lipopolysaccharide (LPS) decreases L-selectin expression of neutrophils (PMNs), thereby preventing PMN-mediated reperfusion injury in the isolated heart. In the present study we determined whether or not that dose of LPS would protect hearts during *in vivo* ischemia and reperfusion by preventing PMN-induced reperfusion injury. Rats receiving saline vehicle showed marked myocardial injury (necrotic area/area at risk = 82% ± 2%) and significant depression in left ventricular function as assessed in the isolated isovolumic heart preparation at constant flow rates of 5, 10, 15, and 20 ml/min. The administration of LPS (100 μg/kg body wt) 7 hr prior to ischemia resulted in a reduction in myocardial damage (necrotic area/area at risk = 42% ± 3%) and preservation of function. Myocardial function was similar to that of sham ischemic saline- and LPS-treated rats. Moreover, PMN infiltration as determined by histology was quantitatively more severe in hearts of saline-treated rats than in hearts of LPS-treated rats. Isolated hearts from vehicle- and LPS-treated animals undergoing sham ischemia *in vivo* recovered to the same extent after *in vitro* ischemia/reperfusion, suggesting that LPS did not induce protection by altering intrinsic properties of the heart. Our results indicate that LPS-induced protection of the heart from *in vivo* PMN-mediated ischemia/reperfusion injury may be due to decreased L-selectin expression of PMNs in LPS-treated animals.

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Key words: endotoxin; LPS; L-selectin; myocardium; rat

During reperfusion of the myocardium following ischemia, two stages of injury can be characterized—early reperfusion injury and neutrophil-(PMN) mediated reperfusion injury. Early reperfusion injury is associated with post-ischemic dysfunction of myocardium and endothelium that occurs within minutes of reperfusion. It may result, at least partly, from an imbalance between the vasodilator and oxygen free radical scavenger nitric oxide (NO) and oxygen-derived free radicals (1–4). However, the occurrence of significant cardiac injury 3 to 4 hr post-reperfusion *in vivo* cannot be explained by the same sequelae.

Subcellular constituents released from irreversibly damaged cells elicit complement activation (5–7), which is responsible for recruitment, chemoattraction, and activation of PMNs associated with reperfusion injury. Moreover, there is a direct correlation between the amount of PMN infiltration and the mass of infarcted tissue (8, 9).

It has been shown that endotoxin, its nontoxic derivatives such as monophosphoryl lipid A, and cytokines induce protection of the heart from ischemia/reperfusion (IR) injury (7, 10–14). Administration of endotoxin (15, 16) or cytokines (17), or induction of gram-negative sepsis (18) has been shown to protect the heart from IR injury 24 hr after the initial insult. Endotoxin increases synthesis of proteins such as the heat shock proteins (e.g., HSP 70) and the antioxidant catalase by the myocardium. These proteins may be involved in protection (10, 19) from IR injury. Sepsis induces NO synthase, which increases NO production. NO has been shown to inhibit PMN adhesion and infiltration (2, 3, 20). Most of these studies of myocardial protection from IR injury have focused on intracardiac mechanisms of protection (10, 19–21). Recent studies have shown that extracardiac mechanisms can also be manipulated to attenuate IR injury.

Endotoxin has been shown to induce a rapid reduction in leukocyte-endothelium interaction (21–24), which is the first step required for infiltration of PMNs from the vascular space into the interstitial space. Both the integrins and L-selectin on PMNs, as well as their counter receptors (e.g., ICAM-1 and ELAM-1, respectively) on the endothelial cells, are important for PMN adhesion. Endotoxin has been shown to alter expression of adhesion molecules on the PMN surface, thereby directly altering binding kinetics (22).

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Recently, we have demonstrated that PMNs obtained from a rat administered a single nonlethal dose of endotoxin have an impaired ability to induce PMN-mediated IR injury in the isolated perfused heart compared with PMNs from saline-treated control rats (25). These *in vitro* studies suggest that injuries in hearts reperfused with PMNs from endotoxin-treated rats are due only to ischemia and nonPMN-mediated reperfusion injury. The impaired ability in PMNs from endotoxin-treated rats to induce injury may result, at least in part, from decreased PMN adhesion to the endothelium of the coronary vasculature. The true test of effective protection from PMN-mediated IR injury, however, is the protection that would occur in the *in vivo* situation. Therefore, we determined if the capacity for PMNs to induce PMN-mediated IR injury *in vivo* would be attenuated in rats treated with a single nonlethal bolus of endotoxin 7 hr prior to regional myocardial ischemia. This is based on the hypothesis that circulating PMNs in endotoxin-treated rats during the neutrophilic stage will have an impaired ability to induce PMN-mediated IR injury *in vivo* as a result of a reduction in L-selectin surface expression.

Methods and Materials

Animal Preparation. Male Sprague-Dawley rats weighing 300 to 350 g were purchased from Charles Rivers Laboratories (Wilmington, MA). They were housed within the animal care facility and given standard rat chow (Purina) and water *ad libitum* for at least 1 week before they were used. Animal use was approved by the Institutional Animal Care and Use Committee.

Rats were anesthetized with ketamine-xylazine (6:0.6 mg/100 g body wt., intramuscularly). Under aseptic conditions, polyethylene catheters (PE 50) were implanted in the carotid artery and the jugular vein. Rats were allowed 24 hr to recover. Rats were given either a nonlethal bolus (100 μg/kg) of LPS (Seratype 0111:B4, Sigma, St. Louis, MO) or sterile saline. The 7-hr time point was chosen for this study because we have previously shown (25) that 100 μg/kg of LPS (Serotype 0111:B4, Sigma) will consistently induce a fever and decrease in blood pressure with both recovering by 7 hr. By 1 hr animals are neutropenic and by 7 hr they are neutrophilic. With this dose of LPS there was no mortality through 48 hr.

***In Vivo* Ischemia.** Seven hours after treatment, *in vivo* regional myocardial ischemia was induced by occluding the left anterior descending (LAD) coronary artery. The occlusion was performed using a modification of the procedure described by Petty et al. (26). Briefly, rats were anesthetized with pentobarbital sodium (45.5 mg/kg, intravenously). The trachea was cannulated and upon penetration of the thoracic cavity, the animal was ventilated with room air under positive end expiratory pressure (2 cm H_2O) with a tidal volume of 1 ml/100 g and a frequency of 55 strokes/min. Supplemental pentobarbital sodium was given as needed to maintain stable anesthesia. A left thoracotomy was performed at the level of the fifth intercostal space. After gently spreading the ribs and retracting the pericardium, a 5.0 silk ligature was placed under the visualized main branch of the LAD coronary artery just inferior to the left atrial appendage. The artery was occluded for 1 hr followed by 3 hr of reperfusion. Time-matched sham-ischemic controls were prepared with a ligature in an identical manner, but without the coronary occlusion. Body temperature was taken prior to treatment, 1 hr post-treatment (LPS-treated groups only), and immediately prior to surgery for coronary artery occlusion. Blood pressure (BP) and heart rate (HR) were measured prior to treatment, immediately prior to surgery, before and after ischemia (or time-matched sham ischemia), and hourly during reperfusion. BP and HR were measured with a P1000B Narco pressure transducer connected to a Narco physiograph (Bio-Systems, Inc., Houston, TX).

Isolated Heart Preparation. After 3 hr of *in vivo* reperfusion (or continued perfusion with time-matched sham controls), hearts were excised and placed in ice-cold Krebs-Henseleit bicarbonate (KHB) buffer (pH 7.4). Hearts were attached to the metal cannula of a water-jacketed Langendorff perfusion apparatus via the aorta and perfusion was initiated with KHB buffer (pH 7.4) gassed with 95%O_2/5%CO_2 and warmed to 37°C (12, 18). A compliant latex balloon tied to the end of a size 4 French dual-lumen catheter was directed through the atrium, into the ventricle, and through the apex of the myocardial wall. A small piece of balloon was tied to the outer surface of the heart and the catheter was tied securely to the left atrial appendage. A Grass stimulator was used to pace the heart at 320 beats/min. A heated water jacket was placed around the heart and an insulating foam cover was set on top of the chamber in order to maintain the chamber temperature at 37°C. A P23 XL pressure transducer (Spectramed, Oxnard, CA) was attached to one lumen of the dual-lumen ventricular catheter and maximum ventricular pressure, left ventricular end diastolic pressure (LVEDP), and positive and negative dP/dt_{max} (measure of rate of left ventricular pressure rise and fall during a cardiac cycle) were recorded by a Grass physiograph. Left ventricular developed pressure (LVDP) was calculated as the difference between systolic and diastolic pressure. The height of the buffer in the Langendorff column was maintained at a level to deliver coronary flow (CF) at a perfusion pressure of 80 mmHg. Coronary flow was measured by collecting coronary effluent for 30 sec as it dripped from the heart. Coronary pressure was determined by a second P23 XL pressure transducer (Spectramed) at the level of the heart attached to the perfusion system via a lateral side-port.

***In Vivo* Ischemia—*in Vitro* Function.** *Constant pressure protocol.* Hearts were stabilized for 15 min with a balloon volume of 100 μL. After a control measurement was taken, the balloon volume was removed and replaced with 25 μL. Hearts were allowed 5 min to re-stabilize and CF and ventricular pressure were recorded. This procedure

was repeated for balloon volumes of 50, 75, and 100 μL, respectively.

Constant flow protocol. Following measurement of ventricular performance under constant pressure perfusion, hearts were switched to a constant flow system and were allowed to stabilize for 5 min with a balloon volume of 100 μL and a CF of 5 ml/min. Coronary and ventricular pressures were recorded. This procedure was repeated for perfusion rates of 10, 15, and 20 ml/min.

***In Vitro* Ischemia.** Following constant flow protocols, hearts from time-matched sham-ischemic, endotoxin- or saline-treated rats were allowed to stabilize for 5 min at a constant perfusion pressure of 80 mmHg. The balloon was filled until ventricular diastolic pressure was 5 mmHg (approximately 100 μL). Following an initial recording of CF and ventricular pressure, hearts were subjected to 30 min of global ischemia and 30 min of reperfusion. The water was removed from the balloon during ischemia in order to maximize myocardial damage upon reperfusion (27). The same volume of water was re-introduced into the balloon immediately upon reperfusion. CF and ventricular pressure were obtained at 10, 20, and 30 min of reperfusion. An additional group of hearts from naïve animals was used to control for possible intrinsic myocardial protection to IR injury induced by surgery and/or the LPS-treatment.

Myocardial Tissue Analysis. Myocardial injury was evaluated by the triphenyl tetrazolium chloride-Evan's Blue technique (26). Following constant flow protocols, the LAD artery was re-occluded in hearts from ischemic groups, and coronary perfusion was performed for 3 min with the addition of 1% Evan's blue dye in KHB buffer to stain the area of the myocardium perfused by the patent coronary arteries. The area at risk was therefore determined by negative staining. The atria were removed and the right ventricle, left ventricle, and septum were separated. The unstained portion of the myocardium (i.e., the area at risk) was separated from the stained portions. All unstained portions were sliced into 1-mm-thick pieces and incubated in 0.1% triphenyl tetrazolium chloride (TTC) in KHB buffer (pH 7.4) at 37°C for 15 min to detect the presence of coenzyme and dehydrogenase. The portion of the myocardium that did not stain, the necrotic region, was separated from the area at risk. Total septal and ventricular weights, area at risk weights, and necrotic tissue weights were determined.

Histology. One heart from each group was randomly chosen for histologic analysis. Directly after the constant flow protocol hearts were fixed in Z-fix (Anatech, Battle Creek, MI) for 8 to 14 days at room temperature prior to paraffin embedding. A set (3–4 sections each) of serial transverse 10-μm-thick sections was obtained at five different levels of the ventricular wall between the base and 1.5 mm from the apex. The serial sections were stained by incubation with α-naphthyl acetate in the presence of Fast Blue RR salt (stable diazonium salt) and counterstained with hematoxylin for analysis and quantification of PMN infiltration. The α-naphthyl acetate esterase enzymatic staining technique was used because of its specificity for PMN localization and cell-type determination. The materials and standardized technique for staining were obtained from a Sigma Diagnostics Kit (Procedure No. 90) for Naphthol AS-D Chloroacetate Esterase and α-Naphthyl Acetate Esterase for paraffin-embedded tissues.

Statistical Analysis. Either a *t* test or a one-way analysis of variance (ANOVA) was used to determine significant differences between two or several groups, respectively. One-way ANOVA with repeated measures was used to determine significant differences between pre-ischemic and reperfusion values within a group. Differences were considered statistically significant at $P < 0.05$.

Results

In isolated hearts perfused at a coronary perfusion pressure of 80 mmHg, LVDP was depressed in hearts from saline-treated rats after a 1-hr *in vivo* coronary artery occlusion and a 3-hr reperfusion (Fig. 1). At the lowest pre-load, 25 μl balloon volume, the saline-ischemia group was significantly different from the LPS-treated ischemia group, the saline-treated sham group, and the LPS sham ischemia group. In contrast, hearts from LPS-treated rats that were made ischemic *in vivo* demonstrated no difference from the sham-ischemic control hearts obtained from either saline-treated or LPS-treated rats. The depression in cardiac performance seen in the post-ischemic hearts of the saline-treated rats occurred despite elevated coronary flow in this group (Fig. 2). As with cardiac performance, no difference was observed in CF between the sham-ischemic control hearts from LPS- and saline-treated rats.

Due to the apparently greater CF in hearts from the saline-ischemic group during constant pressure perfusion, hearts were also perfused under constant flow conditions. When cardiac performance was measured at constant coronary flows of 5, 10, 15, and 20 ml/min, myocardial perfor-

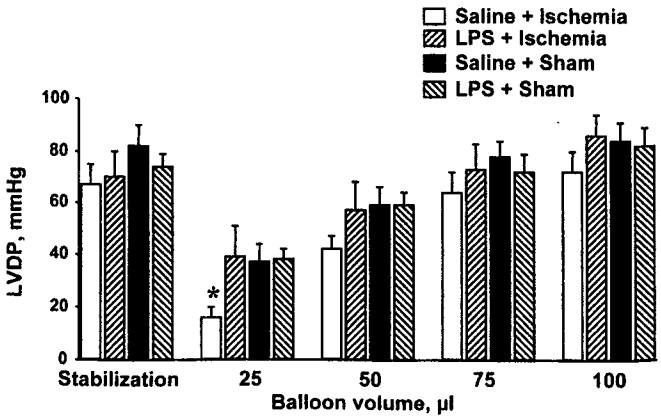

Figure 1. Left ventricular developed pressure (LVDP) in isolated hearts perfused with a constant coronary perfusion pressure of 80 mmHg. Developed pressure is shown at various balloon volumes after a period of stabilization. Hearts were removed from LPS-treated rats or saline-treated rats that underwent 1 hr of ischemia and 3 hr of reperfusion or sham ischemia/reperfusion *in vivo*. *$P < 0.05$, different from other hearts with the same balloon volume.

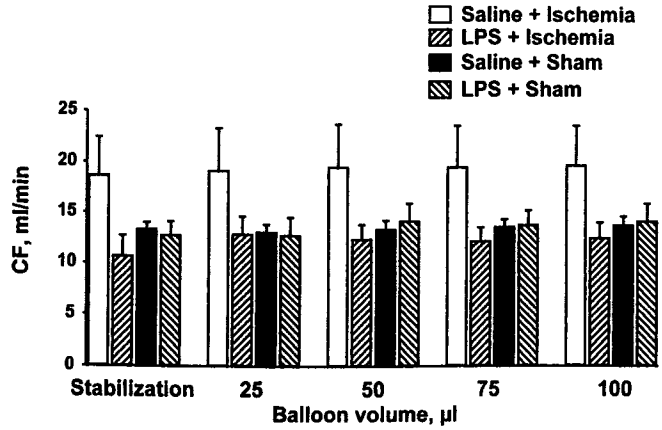

Figure 2. Coronary flow (CF) in isolated hearts perfused with a constant coronary perfusion pressure of 80 mmHg. Coronary flow is shown at various balloon volumes after a period of stabilization. Hearts were removed from LPS-treated rats or saline-treated rats that underwent 1 hr of ischemia and 3 hr of reperfusion or sham ischemia/reperfusion *in vivo*.

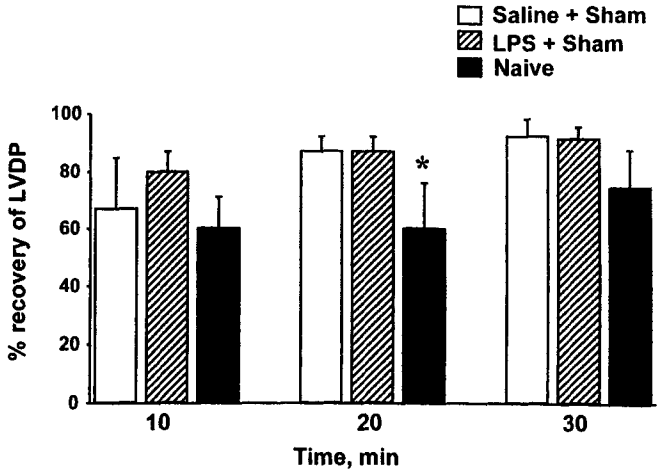

Figure 4. Left ventricular developed pressure (LVDP), as a percent of pre-ischemic LVDP, in isolated hearts reperfused after 30 min of *in vitro* ischemia. Hearts were removed from naïve animals or from LPS-treated rats or saline-treated rats that underwent sham *in vivo* ischemia. $^*P < 0.05$, different from other hearts with the same balloon volume.

mance was not impaired in hearts from LPS-treated rats that had undergone 1 hr of ischemia and 3 hr of reperfusion (Fig. 3). LVDP was similar to that found in the sham-ischemic saline- and LPS-treated rats. However, myocardial performance in the isolated hearts from saline-treated rats that had undergone *in vivo* coronary artery ligation and reperfusion was significantly depressed throughout the entire range of CFs.

In order to determine if arterial and venous catheterization, thoracotomy and placement of the ligature around the left anterior descending artery, and/or administration of endotoxin contributed to myocardial protection by "preconditioning" the heart, hearts from naïve and sham-ischemic rats were made ischemic under *in vitro* conditions. Isolated hearts from naive rats and from sham-ischemic LPS- and saline-treated rats were made globally ischemic for 30 min and were reperfused for 30 min. LVDP, as a percentage of the pre-ischemic LVDP, was similar in the three groups of hearts by 30 min reperfusion, although the hearts from naïve animals required a longer time to recover (Fig. 4).

Myocardial infarction was evident in the coronary artery occluded rat hearts. Pretreatment with LPS caused a significant reduction in infarct size, 42% ± 3% of the area at risk versus 82% ± 2% in the saline-treated group (Fig. 5). The area at risk in the coronary occlusion groups was not different. The percentage of the ventricle undergoing necrosis was greater in the hearts from the saline-treated ischemic rats than in hearts from LPS-treated ischemic rats.

Dynamic compliance was calculated by plotting the left ventricular end diastolic pressure as a function of balloon volume at a constant coronary perfusion pressure. Dynamic compliance was decreased in hearts removed from saline-treated rats that had undergone *in vivo* coronary artery ligation and reperfusion (Fig. 6). In hearts from LPS-treated rats that had undergone *in vivo* myocardial ischemia and

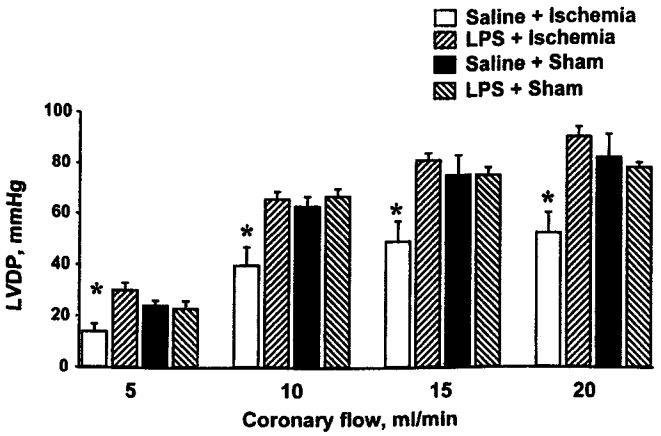

Figure 3. Left ventricular developed pressure (LVDP) in isolated hearts perfused with constant coronary flows of 5, 10, 15, and 20 ml/min. Hearts were removed from LPS-treated rats or saline-treated rats that underwent 1 hr of ischemia and 3 hr of reperfusion or sham ischemia/reperfusion *in vivo*. $^*P < 0.05$, different from other hearts with the same balloon volume.

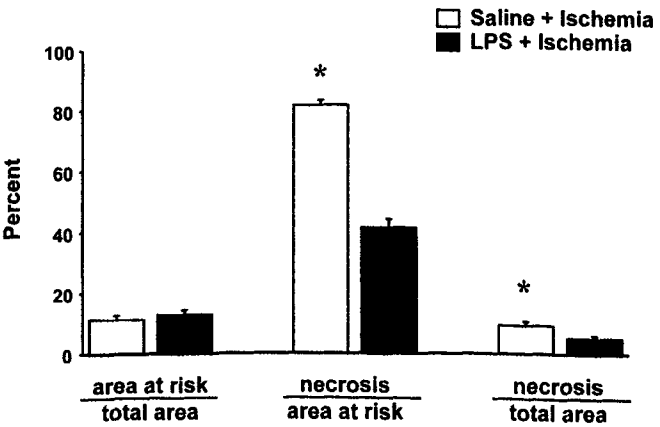

Figure 5. The area at risk as a percentage of the total ventricle, and the necrotic area as a percentage of the area at risk and as a percentage of the total ventricle. $^*P < 0.05$, different from LPS + ischemia.

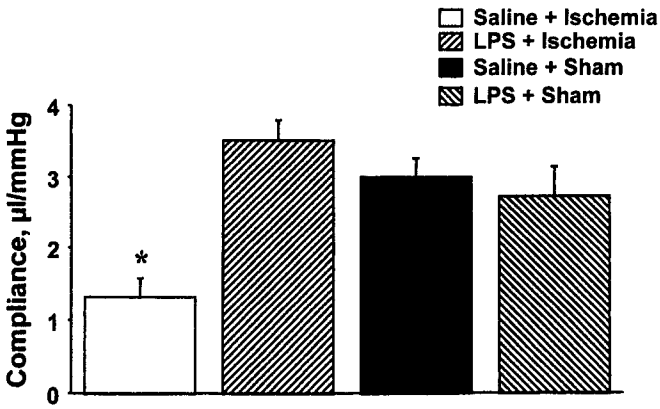

Figure 6. Dynamic compliance of the left ventricle of hearts from LPS- or saline-treated rats exposed to *in vivo* ischemia and reperfusion or sham ischemia. Dynamic compliance was calculated as the slope of the curve generated by plotting balloon volume versus left ventricular end diastolic pressure. *$P < 0.05$, different from other groups.

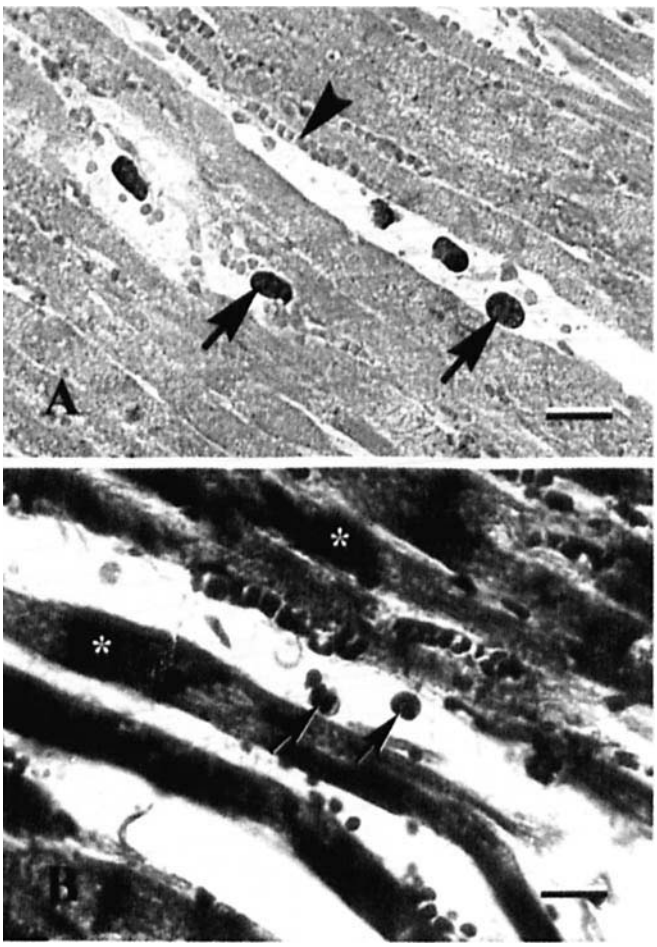

Figure 7. (A) Immunohistochemical staining of a heart from a saline-treated rat that underwent coronary artery occlusion and reperfusion. Extravasated neutrophils are evident. (B) Trichrome staining of the same heart as in (A) demonstrating tissue necrosis as indicated by the presence of contraction bands. Both (A) and (B) were obtained from the region of the myocardium perfused by the left anterior descending coronary artery. Arrows indicate neutrophils; arrowheads indicate capillaries; * = contraction band. Bar represents 20 µM.

Figure 8. Immunohistochemical staining of heart from LPS-treated rat that underwent *in vivo* ischemia and reperfusion. Neutrophils are restricted to the intravascular space. Arrows indicated neutrophils. Bar represents 20 µM.

reperfusion, dynamic compliance was similar to that found in the sham-ischemia groups.

Cross-sectional histological analysis of myocardium perfused by the LAD artery demonstrated that after 1 hr of ischemia and 3 hr of reperfusion *in vivo*, PMNs in a saline-treated rat extravasated and infiltrated the myocardium (Fig. 7A). Areas of ischemic injury and early necrosis were identified by histology within these hearts by the presence of contraction bands (Fig. 7B). In contrast, PMNs visualized within post-ischemic hearts from LPS-treated rats were mainly restricted to the intravascular space (Fig. 8). Sections of sham-ischemic hearts from LPS-treated rats demonstrated that PMNs were limited almost exclusively to the intravascular space (Fig. 9).

Discussion

In 1986, Murray, Jennings, and Reimer first demonstrated with ischemic preconditioning that it was possible to

Figure 9. Immunohistochemical staining of sham-ischemic heart from LPS-treated rat. Neutrophils are restricted to the intravascular space. Arrows indicate neutrophils. Bar represents 20 µM.

induce myocardial protection against ischemia/reperfusion injury (28). Since then, comparable results have been achieved in different animal models employing adenosine (via A_3-receptor activation), norepinephrine, (via α_{1b}-adrenergic receptor activation), acetylcholine, morphine, phospholipase C activators, and various other agents (29–32). The intracellular mechanisms and responses that may mediate protection include activation of ATP-regulated K+ channels and/or a vacuolar proton ATPase; induction of NO synthase (iNOS), and/or heat shock proteins; release of prostaglandins and/or adenosine; and inhibition of mitochondria ATP proteins (30, 33–38). Other approaches to induce myocardial protection have used gram-negative bacteria, LPS, cytokines, or monophosphoryl lipid A (10, 12, 17, 39). Mechanisms of protection have been postulated to include up-regulation of iNOS, catalase, and G-6-PDH, and activation of K_{ATP}-channels (10, 40). Therefore, the primary target of research against IR injury has been focused on intrinsic myocardial protection against ischemia and early reperfusion injury as mediated by oxidative stress. However, protection against PMN-mediated IR injury, particularly through extracardiac mechanisms, has not been thoroughly investigated.

It has been well established since the early 1970's that PMN infiltration into the myocardium following reperfusion of infarcted tissue exacerbates the injury, resulting in greater permanent myocardial compromise (41). Therefore, two main extracardiac strategies can potentially decrease the amount of tissue compromised and/or lead to increased recovery of performance following an IR insult *in vivo*. The first strategy is to decrease the circulating level of PMNs upon time of reperfusion (42). Using a rat heterotopic heart-transplant model of IR injury, Galinanes and associates demonstrated that initially transfusing for 10 min or longer upon reperfusion with blood made leukopenic with mustine hydrochloride accelerated the rate of recovery of cardiac function after 1 hr of reperfusion (43). The second strategy is to transiently compromise the ability of circulating PMNs to extravasate and infiltrate tissue. Pre-treating animals with either sublethal doses of endotoxin or monophosphoryl lipid A (MLA) can markedly reduce endotoxin-induced mortality by inhibiting PMN activation caused by a subsequent lethal endotoxin challenge (44, 45).

Our model of *in vivo* LAD coronary artery occlusion and reperfusion substantiates extracardiac mechanisms by which endotoxin can be protective. In our model, endotoxin limits further injury to post-ischemic tissue. One hour of coronary artery occlusion and 3 hr of reperfusion preserved myocardial performance measured *in vitro* under constant flow if animals were first pre-treated with endotoxin 7 hr prior to occlusion. The heart possesses a substantial degree of myocardial reserve that allows for increased myocardial performance under conditions of increased demand. This reserve may enable the heart to sustain some irreversible damage without compromising myocardial performance under resting conditions. For example, the post-ischemic hearts from the LPS-treated rats had approximately 16% of the left ventricle infarcted and yet demonstrated *in vitro* function similar to that of the sham-ischemic saline- and LPS-treated rats. However, at some degree of injury, myocardial performance is compromised as in the saline-ischemic group. Performance studies alone would suggest that endotoxin *in vivo* could protect the heart from reperfusion injury and limit the amount of damage to which these hearts would be subjected.

Heart function studied under constant pressure perfusion demonstrated two subtle findings. First, only at the lowest preloads did myocardial performance appear to be compromised in the hearts from saline-treated rats made ischemic *in vivo*. Second, CFs tended to be elevated in these hearts during constant pressure perfusion. Therefore we assessed myocardial performance under constant CF conditions.

With constant CF rates, myocardial performance was consistently depressed in ischemic hearts from saline-treated rats relative to sham-ischemic controls. In contrast, myocardial performance in ischemic hearts from endotoxin-treated animals was spared and could not be differentiated functionally from sham controls. The reason for the apparent discrepancy in myocardial performance between constant flow and constant pressure protocols for the ischemic hearts from saline-treated rats may be attributed to the "Gregg phenomenon" (46). Under constant pressure perfusion, CFs were elevated in the ischemic hearts from rats pre-treated with saline. The Gregg phenomenon, sometimes explained as the "garden-hose effect" or by the coronary distention hypothesis, suggests that as the coronary wall dilates in response to the increased CF, the stretched wall also elongates the adjacent myocytes, resulting in increased systolic function and oxygen consumption (47).

The decreased performance in post-ischemic hearts from saline-treated rats under constant flow conditions may reflect increased amounts of akinetic tissue and/or decreased compliance of the myocardial wall. The decreased compliance (increased stiffness) of the myocardium has been directly linked with increased myocardial injury, although the greater CF in this group may also contribute to the decreased compliance. Other models of myocardial ischemic injury have demonstrated decreased ability of injured myocytes to rapidly remove calcium from the cytosol (48). Acute decreases in myocardial compliance are closely linked with cytosolic calcium overload and associated cytological abnormalities. Furthermore, an increased fraction of akinetic myocardium may limit the ability of the heart to contract at lower preloads. Enhanced fluid leakage and vessel injury within the LAD artery distribution may also contribute to decreased compliance and/or decreased function. Decreased myocardial performance coupled with decreased wall compliance in post-ischemic hearts from saline-treated rats suggests that there was a greater degree of myocardial injury and/or infarcted tissue in this group. In contrast, the post-ischemic hearts from the LPS-treated rats matched CF,

as well as myocardial performance and wall compliance, to that seen with sham-ischemic control hearts, implying that there was substantially less injury in post-ischemic hearts from the LPS-treated group. The *in vitro* ischemia studies demonstrating that hearts from saline- and LPS-treated rats recovered to the same extent suggest that LPS treatment did not endow any degree of intrinsic myocardial protection to account for the better recovery in the LPS-treated group.

When the amount of myocardial tissue injured was evaluated by the TTC-Evan's Blue technique, a greater proportion of myocardium was shown to be infarcted in post-ischemic hearts from the saline-treated group than from the LPS-treated group. The 2-fold difference in the amount of infarcted myocardium verifies the conclusions made from the performance data. Other investigators have demonstrated that LPS and MLA pre-treatment can reduce the amount of infarcted tissue after ischemia (11, 49). Eising and associates (11) proposed that LPS might induce a general nonspecific down-regulation of the inflammatory response. We recently demonstrated that PMNs isolated from LPS-treated rats during the neutrophilic stage of endotoxemia have an impaired ability to induce PMN-mediated IR injury in the isolated heart (25). These studies showed that under *in vitro* conditions, PMNs from LPS-treated rats had a substantially decreased capacity to infiltrate the myocardium. In the presently established model for PMN aggregation and adhesion, both lectin and integrin adhesion molecules are required sequentially (50, 51). Therefore, the inhibition of PMN aggregation after exposure to LPS suggests that LPS may affect the balance between aggregation and adhesion by shedding L-selectin receptors (22). Studies comparing CD11b/CD18 and L-selectin receptor density versus PMN aggregation have verified that the ability of PMNs to aggregate decreases irrespective of the CD11b/CD18 density (21–24, 52). Moreover, PMNs obtained 7 hr after the administration of 100 μg/kg LPS had a 50% reduction in L-selectin receptor expression compared with PMNs from saline-treated controls (25). Histological analysis of myocardium perfused by the LAD artery substantiates the theory that LPS retards the ability of PMNs under *in vivo* conditions, as well as under *in vitro* conditions, to extravasate and infiltrate injured myocardium because of the decreased L-selectin surface density.

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