Platelets Protect Against Myocardial Dysfunction and Injury Induced by Ischemia and Reperfusion in Isolated Rat Hearts

B.C. Yang, R. Virmani, W.W. Nichols, and J.L. Mehta

Platelets are a source of vasoactive mediators that regulate vascular tone. Platelets also play a role in intravascular thrombus formation and dynamic coronary constriction that result in myocardial ischemia. However, effects of platelets on myocardial function after ischemia and reperfusion are unknown. In this study, we examined the effects of platelets on myocardial dysfunction caused by ischemia/reperfusion. Buffer-perfused isolated rat hearts, after global ischemia (15 minutes) and reperfusion (10 minutes), developed marked myocardial dysfunction, indicated by a 65±4% decrease in the force of cardiac contraction (FCC) and a 26±7% increase in coronary perfusion pressure (CPP). Ischemia/reperfusion was also associated with release of creatine kinase (CK) and ATP metabolites in the coronary effluents. Perfusion of hearts with buffer containing washed rat platelets (3–8×10^7 cells/ml) protected hearts against dysfunction from ischemia/reperfusion, indicated by minimal changes in CPP (−1±1%) and FCC (−1±3%). Release of CK in the coronary effluent was also reduced, as was the release of ATP metabolites in the platelet-perfused hearts. Perfusion of hearts with serotonin receptor antagonist LY53,857 (10^-4 M), thromboxane A2 receptor antagonist SQ29,548 (10^-6 M), adenine nucleotide scavenger apyrase (0.4 units/ml), or nitric oxide synthetase inhibitor N^ω-monomethyl-L-arginine (2×10^-4 M) attenuated (p<0.05) the platelet-mediated cardioprotective effects. Perfusion of the hearts with L-arginine (2×10^-4 M) instead of platelets also showed modest protective effects on FCC (−4.3±13%), CPP (+18±7%), and CK release. Prolongation of the ischemic period to 30 minutes and reperfusion to 20 minutes also demonstrated marked cardiac dysfunction (FCC, −58±10%; CPP, +36±8%) in buffer-perfused hearts. Perfusion of hearts with platelets in this setting of prolonged ischemia/reperfusion also exhibited protective effects on FCC (−24±10%), CPP (+12±6%), and CK release. Thus, platelets protect myocardium from ischemia/reperfusion–induced injury, and these protective effects of platelets are evident regardless of the duration of ischemia/reperfusion. Furthermore, these cardioprotective effects of platelets seem to be related to the release of serotonin, thromboxane A2, and adenine nucleotides. These substances most likely elicit release of endothelium-derived relaxing factor, with its attendant tissue-protective effects, from the microvascular endothelium of hearts. (Circulation Research 1993;72:1181–1190)

KEY WORDS • ADP • endothelium • myocardial ischemia • platelets • thromboxane • serotonin

Platelets are a major source of vasoactive mediators, such as thromboxane (Tx) A2, serotonin, and adenine nucleotides.1 These substances participate in the regulation of vascular tone and thereby influence the circulatory state in physiological and pathological states. Aggregating platelets relax pre-contracted blood vessels with intact endothelium by eliciting the release of endothelium-derived relaxing factor (EDRF).2 The release of EDRF was initially attributed to adenine nucleotides derived from aggregating platelets.2,3 Subsequent studies have suggested that TxA2 and serotonin also stimulate EDRF release.4,5 In the absence of functional endothelium, platelets cause a marked contraction of blood vessels via TxA2 and serotonin-dependent mechanisms.2

A number of studies have suggested that platelets participate in the initiation and propagation of myocardial ischemia. For example, platelets accumulate in the narrowed coronary arteries6,7 as well as in the myocardial tissues8 in animal models of myocardial ischemia. Aggregating platelets also enhance the contraction of blood vessels during anoxia.9 Adhesion of platelets to the subendothelial layers in coronary arteries with atherosclerosis and release of TxA2 and serotonin have been thought of as an initial event in the genesis of occlusive thrombus leading to acute myocardial ischemia.9,10 Similar platelet deposition occurs at the site of coronary angioplasty and may be the basis of acute occlusion of the affected coronary artery. The major denominator for all these conditions in which platelets seem to play a pathological role is defective endothelial
function. A recent study shows that platelets influence cardiac papillary muscle tone depending on the presence of endocardium.\textsuperscript{11} On the basis of several of these observations,\textsuperscript{8,9,10} Vanhoutte and Houston\textsuperscript{12} first proposed an important interaction between platelets and blood vessel walls in the induction of vasospasm and precipitation of myocardial ischemia.

In animals subjected to coronary artery occlusion followed by reperfusion, myocardial tissues develop tissue injury that has been attributed to the release of free oxygen radicals\textsuperscript{13,14} and accumulation of neutrophils in the reperfused tissues.\textsuperscript{15,16} Although neutrophil accumulation and activation may increase coronary vascular tone\textsuperscript{17} and the extent of tissue injury,\textsuperscript{18} the role of platelets in ischemia/reperfusion-mediated injury remains largely unknown.

The present study was designed to examine the role of platelets in injury caused to the myocardium by a period of prolonged ischemia and reperfusion. These studies were conducted in isolated perfused rat hearts to exclude the confounding effect of other circulating cells.

Materials and Methods

Isolated Perfused Heart

Male Sprague-Dawley rats weighing approximately 250 g were anesthetized with xylazine (60 mg/kg) plus ketamine HCl (150 mg/kg) intraperitoneally. Whole blood was drawn from the common carotid artery to harvest platelets. The hearts were rapidly excised and placed in ice-cold Krebs-Henseleit (K-H) buffer composed of (mM) NaCl 118, KCl 4.7, KH\textsubscript{2}PO\textsubscript{4} 1.2, MgSO\textsubscript{4} 1.2, CaCl\textsubscript{2} 2.5, NaHCO\textsubscript{3} 25, and glucose 11. Within 1 minute, the hearts were transferred to a perfusion apparatus and perfused via the aorta with oxygenated (95% O\textsubscript{2}, 5% CO\textsubscript{2}) buffer solution kept at 37°C, pH 7.4, using a MasterFlex pump (model 7016-21, Cole-Palmer Instrument Co., Chicago, Ill.) according to the modified Langendorff procedure.\textsuperscript{14,15} The hearts were allowed to beat spontaneously and were perfused with K-H buffer at constant flow (4 ml/min). Coronary perfusion pressure was measured via a catheter placed just proximal to the coronary arteries and connected to a Gould Statham P23ID pressure transducer. To measure the force of cardiac contraction, the base of the heart was fixed, and a metal hook was attached to the apex of the heart and connected to a force transducer (Kristler Morse, Redmond, Wash.) and a lever system, as described recently by us.\textsuperscript{20} The preload before any intervention was kept at 2 g. Both the coronary perfusion pressure and cardiac contractile force were continuously recorded on a four-channel recorder (Astro-Med, Inc., West Warwick, R.I.). Heart rate was calculated from a fast-speed tracing of the cardiac contraction signal.

Platelet Preparation

Rat blood withdrawn from the carotid artery was collected in 3.8% sodium citrate (9:1) and centrifuged at 150g for 10 minutes at 20°C to obtain platelet-rich plasma, which was centrifuged again at 1,000g for 20 minutes. Platelets were washed with Tris-sodium glucose (TSG) buffer composed of (mM) Tris-HCl 15, NaCl 134, glucose 5, and EDTA 1 (pH 7.4) and suspended in TSG buffer.\textsuperscript{21} These platelets aggregated normally in response to calcium ionophore A23187 (10 µM).

Experimental Protocol

Four hearts were continuously perfused for 45 minutes and served as sham controls for any spontaneous changes in cardiac dynamics and creatine kinase (CK) release. In the brief ischemia/reperfusion group, hearts were allowed to equilibrate for 20 minutes and were then subjected to 15 minutes of total ischemia (stop perfusion) and 10 minutes of reperfusion. Eleven hearts were perfused with K-H buffer (4 ml/min) and platelets (in a volume of 0.2 ml/min given through a separate side arm just proximal to the aortic cannula). The final concentration of platelets was 3×10\textsuperscript{10} (n=15) and 8×10\textsuperscript{11} cells/ml (n=6). Ten minutes later, these hearts were subjected to 15 minutes of total ischemia followed by 10 minutes of reperfusion. Platelets were perfused throughout the period of reperfusion.

To investigate the mechanism of the effects of platelets on indexes of myocardial injury, some hearts (n=6 each) were perfused with the ATP/ADP scavenger apyrase (0.4 units/ml), the serotonin (5HT) receptor antagonist LY53,857 (10\textsuperscript{-6} M), the TxA\textsubscript{2} receptor antagonist SQ29,548 (10\textsuperscript{-6} M), or the nitric oxide synthetase inhibitor N\textsuperscript{\omega}-monomethyl-L-arginine (L-NMMA, 2×10\textsuperscript{-4} M) along with platelets (3×10\textsuperscript{11} cells/ml). These hearts were then subjected to 15 minutes of ischemia followed by 10 minutes of reperfusion. In some experiments (n=6 each), hearts were perfused with apyrase, LY53,857, SQ29,548, and L-NMMA without platelets to determine the direct effects of these agents on cardiac dynamics. Some hearts (n=6) were perfused with nitric oxide precursor L-arginine (2×10\textsuperscript{-4} M) instead of platelets and then subjected to 15 minutes of ischemia followed by 10 minutes of reperfusion.

Coronary effluent was collected before ischemia and several times during reperfusion and placed in ice for CK measurement. Coronary effluent was also collected for measurement of ATP metabolites before ischemia and during reperfusion, kept in shaking water bath at 100°C for 5 minutes, and then saved at -4°C. Effluents were also collected for measurement of 6-ketoprostaglandin F\textsubscript{1a} (6-keto-PGF\textsubscript{1a}) release. After completion of the experiment, hearts were saved at -70°C for subsequent measurement of CK; some hearts (n=6) perfused with buffer or platelets were perfused with Trump-McDowell fixative for 5 minutes and saved in the fixative for subsequent electron microscopic examination.

To determine the effects of platelets on prolonged myocardial ischemia and reperfusion, another group of hearts (n=6) were perfused with buffer or platelets and subjected to 30 minutes of ischemia followed by 20 minutes of reperfusion.

CK Measurement

Myocardium was homogenized in distilled water. The homogenate was centrifuged at 5,000g for 15 minutes at 4°C. The CK activity in the supernatant and in the
coronary effluent was measured spectrophotometrically. Supplies for CK assay were obtained from Sigma Chemical Co., St. Louis, Mo. (CK kit No. 45-UV). CK in the coronary effluent was measured within 4 hours after collection.

6-Keto-PGF₁α Assay

Prostaglycin (PGI₁) release was measured in the coronary effluents by quantitation of its stable hydrolysis product 6-keto-PGF₁α by enzyme-linked immunosorbent assay (ELISA), as described earlier. The supplies for ELISA were obtained from Cayman Chemical Co. Inc., Ann Arbor, Mich.

Measurement of ATP Metabolites

Coronary effluent samples were assayed for hypoxanthine, inosine, and adenosine by reverse-phase high-performance liquid chromatography (HPLC) according to the method used by Hartwick et al. The HPLC system included an injector (model 710B, Waters, Milford, Pa.), an LKB 2150 pump (Pharmacia LKB, Bromma, Sweden), and an absorbance detector (model 160, Beckman Instruments, Palo Alto, Calif.). Samples were passed through an ultraspHERE ODS (5-μm) column (Alltech, Palo Alto, Calif.) containing gradient medium (20 mM KH₂PO₄ [pH 5.7] and 20% methanol [vol/vol]). The hypoxanthine, inosine, and adenosine peaks were detected at 254 nm, recorded on a strip-chart recorder (model BD40, Kipp & Zonene, Bohemia, N.Y.), and integrated with a 3392A integrator (Hewlett-Packard Co., Palo Alto, Calif.).

Transmission Electron Microscopy

Buffer-perfused and platelet-perfused hearts were subjected to transmission electron microscopy (TEM) as described earlier. Briefly, tissue samples were taken from the endocardial and epicardial regions of the left ventricle and cut into 1-mm cubes fixed in 3% buffered glutaraldehyde for TEM. Tissues were allowed to fix for 1–6 hours and then were transferred to 1% osmium tetroxide in 0.1 M cacodylate buffer, dehydrated, and embedded in Epon. The artifact-free areas with the most capillaries were selected for ultrathin section cutting and examined with Zeiss 1091 GF electron microscope.

Reagents

[1S-[1α,2β(5Z),3β,4α]-7-[3’-[2-[((Phenylamino)carbonyl)hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid, the TXA₂-endoperoxide receptor antagonist SQ29,548, was a gift of Squibb, Princeton, N.J. 4-Isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl)-4,6,6a,7,8,9,10,10a-octahydroadolol(4,3-FD)quinoline maleate, the S₄ receptor antagonist LY53,857, was obtained from Eli Lilly and Co., Indianapolis, Ind. All other supplies were purchased from Sigma.

Statistical Analysis

Changes in coronary perfusion pressure, force of cardiac contraction, and heart rate are expressed as percent increase or decrease from preischemic values. All values are expressed as mean±SEM. One-way and two-way analyses of variance were used to evaluate the statistical significance. A value of p<0.05 was considered significant.

Results

Cardiac Dynamics During Ischemia and Reperfusion

In the control continuously perfused hearts observed for 45 minutes, there were only minimal changes in coronary perfusion pressure, the force of cardiac contraction, and heart rate. In hearts subjected to a brief period of global ischemia (15 minutes) followed by reperfusion (10 minutes), the force of cardiac contraction decreased 65±4% (p<0.001 versus the control group), coronary perfusion pressure increased 26±7% (p<0.001), and the heart rate decreased 65±5% (p=NS) after 10 minutes of reperfusion (Table 1). These changes in the force of cardiac contraction and coronary perfusion pressure reflect marked myocardial dysfunction.

Hearts perfused with platelets demonstrated a modest (19±4%), but consistent, increase in the force of cardiac contraction, without any change in coronary perfusion pressure or heart rate. After 15 minutes of ischemia and 10 minutes of reperfusion, the force of cardiac contraction, coronary perfusion pressure, and heart rate were unchanged from values just before the onset of ischemia. However, the values of coronary perfusion pressure and the force of cardiac contraction after ischemia and reperfusion were markedly (p<0.001) different from those in hearts perfused with buffer alone (Table 1). Thus, the
platelet-perfused hearts did not demonstrate the adverse effect of 15 minutes of ischemia and 10 minutes of reperfusion.

Representative examples of cardiac dynamics in hearts subjected to ischemia and reperfusion perfused with and without platelets are shown in Figure 1, and data from multiple experiments are presented in Figure 2. The cardioprotective effects of platelets were observed at both concentrations (3×10⁷ and 8×10⁷ cells/ml) of platelets and were similar.

Hearts subjected to prolonged periods of ischemia (30 minutes) and reperfusion (20 minutes) showed a marked decrease in the force of cardiac contraction (−58±10%) and a marked increase in coronary perfusion pressure (+36±8%). Hearts perfused with platelets and subjected to prolonged ischemia and reperfusion showed only modest deterioration in the force of cardiac contraction (−24±10%) and a smaller rise in coronary perfusion pressure (+12±6%). These changes in cardiac dynamics in platelet-perfused hearts were

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**Figure 1.** Representative recordings showing cardiac dynamics in a rat heart perfused with buffer and subjected to 15 minutes of ischemia and 10 minutes of reperfusion (top panel). Note the marked decrease in the force of cardiac contraction and increase in coronary perfusion pressure. These changes were not observed in a rat heart perfused with platelets but subjected to similar duration of ischemia and reperfusion (bottom panel).

**Figure 2.** Graphs summarizing changes in the force of cardiac contraction and coronary perfusion pressure in buffer-perfused (n=17) and platelet-perfused (n=21) hearts subjected to brief periods of ischemia (15 minutes) and reperfusion (10 minutes) (left panels) and others subjected to prolonged periods of ischemia (30 minutes) and reperfusion (20 minutes) (n=6 each) (right panels). In both settings, platelet-perfused hearts exhibited less (*p<0.01) decline in the force of cardiac contraction and a smaller (*p<0.01) rise in coronary perfusion pressure than did buffer-perfused hearts. All data points reflect mean±SEM.
significantly less marked than in the buffer-perfused hearts (Table 1 and Figure 2). Since rats in the 15-minute ischemia group and 30-minute ischemia group came from different batches, the data between these two groups are not comparable.

**Mechanism of Cardioprotective Effects of Platelets**

Perfusion of hearts with the S<sub>2</sub> receptor antagonist LY53,857, the TxA<sub>2</sub> receptor antagonist SQ29,548, or the ATP/ADP scavenger apyrase had no effect on cardiac dynamics. However, perfusion of hearts with these agents along with platelets attenuated the platelet-mediated protective effects on cardiac dynamics after 15 minutes of ischemia and 10 minutes of reperfusion. Perfusion of hearts with L-NMMA resulted in a minimal increase of coronary perfusion pressure (8±2%, p=NS). Subsequent perfusion of these L-NMMA–treated hearts with platelets failed to show cardioprotection from ischemia/reperfusion–mediated dysfunction (Figures 3 and 4).

Perfusion of hearts with L-arginine instead of platelets resulted in a modest increase in the force of cardiac contraction (21±6%) without any change on coronary perfusion pressure and heart rate. After 15 minutes of ischemia followed by 10 minutes of reperfusion, decreases in the force of cardiac contraction and increases of coronary perfusion pressure were significantly less (p<0.05) than those in buffer-perfused hearts (Figures 3 and 4).

**CK Release in Coronary Effluents and Myocardial CK Content**

During 45 minutes of continuous perfusion with K-H buffer, there was minimal insignificant CK release in the coronary effluent. In hearts subjected to 15 minutes of total ischemia and 10 minutes of reperfusion, CK was released in the coronary effluents, and the peak level was approximately sevenfold above the normal value (p<0.001) during reperfusion. Perfusion of hearts with platelets (both 3×10<sup>7</sup> and 8×10<sup>7</sup> cells/ml) or L-arginine significantly (p<0.01) attenuated the ischemia-induced CK release (Figure 5). In groups of hearts subjected to prolonged ischemia and reperfusion, perfusion with platelets showed the same protective effect on CK release (data not shown).
The CK content of normal hearts was 247±10 IU/g. Brief periods of total ischemia (15 minutes) and reperfusion (10 minutes) caused a significant reduction in the CK content of hearts (227±7 IU/g, p<0.02). On the other hand, treatment of hearts with platelets resulted in preservation of myocardial CK (257±9 IU/g, p<0.05). Perfusion of hearts with LY53,857, SQ29,548, or apyrase along with platelets abolished the platelet-mediated preservation of myocardial CK activity when hearts were subjected to ischemia and reperfusion (Figure 6).

**PGI₂ Release in Coronary Effluents**

Fifteen minutes of ischemia and 10 minutes of reperfusion caused marked release of PGI₂ as identified by 6-keto-PGF₁α in the coronary effluents during reperfusion. Coronary effluents of platelet-perfused hearts subjected to ischemia and reperfusion also revealed release of PGI₂; however, the magnitude of PGI₂ release in coronary effluents was similar whether hearts were perfused with or without platelets (Figure 7).

**Release of ATP Metabolites in Coronary Effluents**

There was minimal release of ATP metabolites in the coronary effluents of hearts perfused continuously with buffer. Fifteen minutes of ischemia resulted in marked release of hypoxanthine, inosine, and adenosine in the coronary effluents during reperfusion. The maximal release was seen during the first 2 minutes of reperfusion. The release of ATP metabolites was still present at 10 minutes of reperfusion, although at a lower rate (Table 2). In platelet-treated hearts subjected to 15 minutes of total ischemia, peak levels of ATP metabolites were not different from those observed in the hearts perfused with buffer alone. However, the value of all ATP metabolites at 10 minutes of reperfusion was lower, indicating a shorter duration of release of aden-
platelets protect myocardium from ischemia/reperfusion–induced dysfunction and injury and that these protective effects of platelets are related to release of TxA2, serotonin, and adenine nucleotides during interaction between platelets and coronary vasculature.

Platelet aggregation has been observed in the narrowed coronary arteries6,7 as well as in ischemic/reperfused myocardium.8 Antiplatelet agents such as aspirin, TxA2 synthetase, receptor antagonists, and serotonin receptor antagonists have all been shown to decrease intracoronary platelet deposition and to limit myocardial infarct size in experimental models of myocardial ischemia.8,25–29 On the basis of these studies, it has been generally believed that platelets exert a detrimental effect on myocardial ischemic injury via release of TxA2 and serotonin.30,31 However, the present studies indicate that platelets may play an entirely different and cardio-protective role in reperfusion-induced cardiac dysfunction and injury.

We determined cardiac injury by measurement of CK in the coronary effluents as well as residual CK in the myocardium. CK determination has been previously established to reflect the extent of cardiac injury.32 We found diminished CK release in the coronary effluents...
and greater residual CK in the hearts perfused with platelets. These observations suggest that cardiac injury is reduced in hearts perfused with platelets before and after global ischemia.

Release of ATP metabolites has been thought to reflect ATP depletion during myocardial injury.43,44 In our studies, the levels of hypoxanthine, inosine, or adenosine were markedly elevated in the coronary effluents during reperfusion, when the release of ATP metabolites is maximal.43 The peak values of ATP metabolites were similar in hearts perfused with or without platelets (Table 2). However, at 10 minutes of reperfusion, the release of all ATP metabolites was lower in the platelet-perfused hearts than in the buffer-perfused hearts, although only the value for adenosine was significantly lower. The failure to achieve significance with lower values for inosine or hypoxanthine probably reflects a type II error. Nonetheless, the sum of all ATP metabolites was significantly lower in the platelet-perfused (versus buffer-perfused) hearts. The observation of less pronounced ATP depletion in platelet-perfused hearts also suggests a lesser degree of ischemic injury. Although adenosine in large amounts given by intracoronary route is thought to be cardioprotective during myocardial ischemia and reperfusion,34 the concentration of adenosine in the myocardium is too low to provide cardioprotection in the present studies.

TEM showed aggregated platelets in most capillaries of platelet-perfused hearts but not in buffer-perfused hearts. However, perfusion of hearts with platelets had no effect on cardiac dynamics before ischemia except for a modest increase (19±4%) in the force of cardiac contraction.45 Allotti et al46 have recently reported that perfusion of isolated rabbit hearts with autologous platelets plus platelet-activating factor results in reductions in coronary flow, contractile force, and action potential duration and causes arrhythmias. The disparate results of these studies indicate that, depending on the presence or absence of an exogenous stimulus (e.g., platelet-activating factor), platelets may influence cardiac dynamics differently under basal conditions.

Houston et al47 first reported that canine and human platelets cause a concentration-dependent contraction of precontracted large epicardial canine coronary artery rings without intact endothelium via TxA2- and serotonin-dependent mechanisms. This observation has now been confirmed in arteries from several animal species,36–39 in intact animals with coronary artery stenosis and endothelial injury,40 and recently in humans with endothelial injury.41 Shah et al41 exposed cat papillary muscle to homologous washed platelets (3X10^4 cells/ml) and observed that platelets caused contraction of the papillary muscle, which was greater in papillary muscles with intact endothelium. Our study shows that perfusion of hearts with platelets increases the force of cardiac contraction modestly before ischemia but markedly prevents the decrease in the force of cardiac contraction after ischemia and reperfusion. It is possible that the rapid recovery in the force of cardiac contraction after myocardial ischemia relates to the direct isotropic effect of platelets on myocardium. Platelets release several substances, including serotonin, TxA2, adenosine metabolites, and calcium.42,43 Both serotonin and ATP cause contraction of isolated cardiac tissues in vitro.44 Amplification of cardiac responses to ATP by serotonin and other platelet-released substances43,44 and possible changes in calcium concentration and pH during transit of platelets through the heart probably account for the platelet-mediated recovery in the force of cardiac contraction soon after ischemia and reperfusion. This hypothesis is supported by our observations that the S2 receptor antagonist LY53,857, the TxA2 receptor antagonist SQ29,548, and the ATP/ADP scavenger aipyrase all attenuated the myocardial protective effects of platelets.

Adenine nucleotides, TxA2 endoperoxides, and serotonin have all been shown to induce the release of EDRF from blood vessels with intact endothelium.2,45 We hypothesize that the release of large amounts of EDRF from the coronary vascular endothelium after interaction with platelets most likely accounts for the observed effects of platelets during ischemia and reperfusion. Direct evidence for this hypothesis comes from our experiments in isolated hearts perfused with L-arginine, a precursor of EDRF, and subjected to ischemia and reperfusion. Perfusion of hearts with L-arginine provided a modest, but definite, protection against ischemia/reperfusion-mediated cardiac dysfunction. In addition, in experiments in which hearts were pre-treated with L-NMMA, an inhibitor of EDRF synthesis, and then perfused with platelets, the cardioprotective effects of platelets were abolished. These studies clearly demonstrate that cardioprotection by platelets against reperfusion injury relates to release of EDRF. EDRF has indeed been thought of as a cardioprotective factor,45 and its release under the conditions used in our studies could explain the reduction in myocardial dysfunction and injury caused by ischemia and reperfusion.

Temporary coronary occlusion and reperfusion have been shown to result in the release of superoxide radicals that degrade EDRF.46 Loss of EDRF has been proposed to result in a decrease in coronary flow reserve, myocardial stunning, and arrhythmias, collectively known as "reperfusion injury."47,48 A number of studies in animals and isolated hearts have shown that scavengers of superoxide radicals ameliorate several of the manifestations of reperfusion injury.46,48 Since aggregating platelets are a potent stimulus for EDRF release from functional endothelium,2,3 it is likely that the cardioprotective effects of platelets observed in our studies are due to release of large amounts of EDRF, which may downregulate the production of free oxygen radicals.45 The fact that three different and unrelated agents, apyrase, SQ29,548, and LY53,847, attenuated the effects of platelets suggests that the common mode of action of these agents may be the inhibition of the action of platelet-released ATP/ADP, TxA2 endoperoxides, and serotonin, all of which elicit EDRF release.2,45

Since platelet-released cyclic endoperoxides can be used by endothelial cells to synthesize PG12, we wondered if increased synthesis of PG12 was responsible for the cardioprotection conferred by platelets.7 However, the release of 6-keto-PGF1α in the coronary effluents was similar in hearts perfused with or without platelets. Furthermore, neither apyrase, SQ29,548, nor LY53,857 would be expected to attenuate the beneficial effects of platelets observed in these studies, if these effects were mediated via release of PG12.

A number of studies15,16,47–52 suggest that leukocytes participate in reperfusion-mediated cardiac injury. The
detrimental effects of leukocytes have been demonstrated in isolated hearts and coronary vascular rings as well as in anesthetized animals subjected to coronary artery occlusion and reperfusion. The effects of leukocytes appear to be mediated via release of 5-lipoxygenase products, proteolytic enzymes, and free oxygen radicals. Whereas leukocytes exert cardio depressive effects, the demonstration of the cardioprotective effects of platelets in the present study suggests that platelets may have a role in counteracting some of the noxious effects of leukocytes in the setting of myocardial reperfusion.

In summary, our studies indicate that platelets exert cardioprotective effects when isolated rat hearts are subjected to global ischemia and reperfusion. The cardioprotective effects, which are attenuated by apyrase, SQ29,548, LY53,857, and L-NMMA, seem to be related to the release of EDRF in the microvasculature in response to adenosine nucleotides, TxA2, and serotonin. Accordingly, apyrase, TxA2, and S1 receptor antagonists readily attenuate the beneficial effects of platelets on reperfused cardiac tissues. However, it needs to be recognized that the beneficial effects of platelets described in the study relate to the blood-free model of isolated hearts subjected to ischemia and reperfusion. In clinical situations in which the coronary arteries may have long-standing atherosclerosis or in which the endothelium is injured, especially after fissuring or ulceration of the atherosclerotic plaque, the effects of circulating platelets may be different.

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