Beneficial Effects of Iloprost in the Stunned Canine Myocardium

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The effect of the prostacyclin-mimetic, iloprost, on the reversibly damaged ("stunned") myocardium was studied in barbital-anesthetized, open-chest dogs subjected to 15 minutes of coronary artery occlusion and 3 hours of reperfusion. Regional myocardial segment shortening (%SS) was measured in the subendocardium of nonischemic and ischemic-reperfused areas by sonomicrometry. Iloprost was infused for 30 minutes beginning 15 minutes prior to occlusion (0.05 μg/kg/min, ILO-LOW), 0.1 μg/kg/min, ILO-HIGH) or immediately prior to reperfusion (0.1 μg/kg/min, ILO-REP). %SS in the ischemic-reperfused region recovered to 3% of pretreatment values in the control (saline-treated) group by 3 hours of reperfusion. In contrast, %SS in the iloprost-treated groups was significantly enhanced versus the control group at all times of reperfusion. At 3 hours of reperfusion, %SS recovered to 43% (ILO-LOW), 58% (ILO-HIGH), and 35% (ILO-REP) of pretreatment values. The beneficial effect on functional recovery was significantly greater when iloprost was administered before occlusion versus immediately prior to reperfusion. Thus, part of the salutary effects of iloprost appear to occur prior to and/or during ischemia. Iloprost did not improve collateral blood flow to the ischemic region or myocardial high energy phosphate content at 3 hours of reperfusion. While iloprost significantly decreased mean arterial pressure during ischemia and early reperfusion, the hypotensive action did not appear to play a role in the amelioration of postischemic dysfunction, as precondition treatment with an equihypotensive dose of sodium nitroprusside produced no significant effect on postischemic recovery beyond 5 minutes of reperfusion. Results of in vitro experiments indicated that iloprost had no effect on the xanthine oxidase free-radical generating system including lipid peroxidation. However, iloprost decreased the neutrophil-derived superoxide burst after chemotactic stimulation. This beneficial action may, in part, explain the efficacy of iloprost in enhancing postischemic function of the stunned myocardium. (Circulation Research 1988;62:204-215)

Since the isolation of prostacyclin (PGI₂), numerous studies have been performed concerning its influence on experimental cardiac arrhythmias, myocardial infarction, and postischemic dysfunction. Iloprost (ZK 36 374), a chemically stable analogue of PGI₂, has been shown to possess PGI₂-mimetic actions in a variety of experimental preparations. Iloprost has been demonstrated to preserve ischemia-induced loss of myocardial catecholamines, reduce ST-segment elevation and myocardial creatine kinase depletion after coronary artery occlusion in the cat, attenuate the release of complex lipids from the ischemic rabbit heart, reduce infarct size in rats and pigs, and enhance functional recovery after 20 minutes of coronary occlusion in swine hearts. Because these previous studies with iloprost were performed in vitro or in animal models in which a certain amount of irreversible tissue injury occurred, the results obtained cannot be directly extrapolated to episodes of brief periods of regional ischemia.

Reperfusion after brief periods (5–15 minutes) of myocardial ischemia has been shown to result in a prolonged depression of normal myocardial function, biochemical processes, and changes in ultrastructure despite the absence of myocardial cell necrosis. While the myocardium eventually recovers from these brief episodes of ischemia, the underlying mechanism(s) responsible for the reversible dysfunction remains unknown. In addition, the role of eicosanoids, i.e., PGI₂, PGI₂-mimetics, or thromboxane, has not been investigated in this model.

Thus, one objective of the present study was to determine whether the PGI₂-mimetic, iloprost, enhances the recovery of regional myocardial segment shortening in open-chest, anesthetized dogs subjected to 15 minutes of coronary artery occlusion followed by 3 hours of reperfusion. A second objective was to determine whether an effect of iloprost on regional segment shortening was mediated via changes in myocardial blood flow, hemodynamics, or tissue high energy phosphates. The incidence of ventricular fibrillation upon reperfusion in the absence of iloprost was also investigated. Because iloprost produces a reduction in arterial blood pressure, an equihypotensive dose of sodium nitroprusside was investigated to determine whether hypotension alone improves postischemic dysfunction in the "stunned" myocardium. In a separate series of experiments, iloprost was given immediately prior to and throughout the ischemia-reperfusion period in open-chest dogs.
early reperfusion to determine effects of delayed drug intervention. Finally, the effect of iloprost on xanthine oxidase, lipid peroxidation, and neutrophil-induced superoxide burst after chemotactic stimulation was investigated in vitro to determine whether this compound has a free-radical inhibitory action.

Materials and Methods

General Preparation

Adult mongrel dogs (18-30 kg) of either sex were anesthetized with sodium pentobarbital (15 mg/kg i.v.) and barbital sodium (300 mg/kg i.v.) and ventilated by a Harvard respirator (tidal volume of 15 ml/kg, 10-15 breaths/min) with room air supplemented with 100% O₂. Atelectasis was prevented by maintaining an end-expiratory pressure of 5-7 cm H₂O with a trap. Blood samples were obtained from the right femoral artery and coronary vein draining the left anterior descending coronary artery (LAD) perfusion bed, and the pH, Pco₂, and Po₂ were determined by a blood gas analyzer (Radiometer ABL 2). Arterial blood gas values remained relatively constant throughout the experiment (pH 7.35-7.45; Pco₂ 25-35 mm Hg; Po₂ 100-150 mm Hg). Body temperature was maintained at 38°C with a heating pad.

A double-tipped pressure transducer catheter (PC 771, Millar, Houston, Texas) was inserted into the aorta and left ventricle via the carotid artery to monitor mean aortic and left ventricular pressures. The left ventricular pressure pulse was electronically differentiated to obtain left ventricular dP/dt. The right femoral vein was cannulated for administration of drug, vehicle, or subsequent anesthesia as needed. A catheter (Cordis angiographic catheter, 7F, 55-cm length) was inserted into the jugular vein and advanced into the coronary vein adjacent to the LAD such that the tip lay within the ischemic region.

A left thoracotomy was performed at the fifth intercostal space, the lungs retracted, the pericardium incised, and the heart suspended in a pericardial cradle. A 1.0-1.5-cm segment of the LAD was dissected free from surrounding tissue distal to the first diagonal branch and a calibrated electromagnetic flow probe (SP7515 Statham, Oxnard, California) placed around the vessel. Coronary blood flow was measured with a flowmeter (Statham 2202). A micrometer-driven mechanical occluder was placed distal to the flow probe such that there were no branches between the probe and occluder. The occluder was used to zero the flow probe and later occlude the artery. Heart rate was monitored using limb lead II from the electrocardiograph and a tachograph (model 7P4F, Grass Instrument Co., Quincy, Massachusetts). All hemodynamics were monitored on a Grass Model 7 Polygraph.

Myocardial Segment Shortening

Myocardial segment function was measured in the regions perfused by the LAD and left circumflex artery (LCX) by two sets of piezoelectric crystals inserted 7-9 mm into the subendocardium. Crystal depths were verified at the end of each experiment. The leads of the crystals were connected to an ultrasonic amplifier that transforms the crystal-transmitted sound pulse into an electrical signal proportional to the distance between them. The tracings were monitored with an oscilloscope (model 520, Soltec, San Fernando, California). The distance between the two crystals was measured by recording changes in transmission time. Diastolic segment length (DL) was determined at the beginning of the rise phase of positive dP/dt (onset of isovolumetric contraction), and systolic segment length (SL) was determined at peak negative dP/dt. The percent segment shortening (%SS) was calculated using the equation %SS = (DL - SL)/DL × 100. The segment length data were normalized by using a value of 10.0 for the control DL by the method of Theroux et al.

Myocardial Blood Flow

Transmural myocardial blood flow was determined by the radioactive microsphere technique (15-µm spheres) as described previously. Briefly, 10–20 µCi of 147Ce, 51Cr, 103Ru, or 95Nb (approximately 2–4 × 10⁶ spheres) was injected into the left atrium followed by a 6-ml saline flush. Prior to microsphere administration, a collection of reference blood flow from the right femoral artery was begun at the rate of 6.8 ml/min and maintained for 3 minutes.

At the completion of each experiment, India ink was injected into the LAD at the point of the flow probe to delineate the perfusion area. The heart was removed and stored overnight in 10% formalin. The heart was sectioned into tissue pieces from the ischemic and nonischemic areas. Only pieces within at least 2 cm of the black-dyed area were included in data analysis to ensure that tissue with potential overlap flow was not included in the determination of collateral blood flow. All pieces from the center of the ischemic zone were used in the calculation of collateral flow. Each of the pieces (0.5–1.0 g) was sectioned into subepicardium, midmyocardium, and subendocardium. The area-at-risk weight and left ventricular weight were also determined. Tissue and reference blood flow samples were counted in a gamma counter (Searle Analytic 1195).

Myocardial blood flow was calculated using a preprogrammed computer (Apple Ile) to obtain the true activity of each isotope in individual samples. Tissue blood flow was calculated by the following equation: Qm = Qr × Cm/Cr, where Qm is myocardial blood flow (ml/min/g), Qr is rate of withdrawal of reference blood

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FIGURE 1. Structure of iloprost (2Z 36 374).
flow sample (6.8 ml/min), Cr is the activity of the reference blood flow sample (cpm/min), and Cm is activity of the tissue sample (cpm/g). Transmural blood flow was calculated as the weighted average of the three layers in each region. The mean endocardial-to-epicardial (endo/epi) flow ratio was also determined.

Metabolism Biopsy

Immediately prior to killing at 3 hours postreperfusion, a small area (1.5 cm) in normal and postischemic regions was painted with methylene blue dye. Transmural tissue samples were obtained at the dye sites by the use of a cylindrical cutting tool mounted on a hand drill as described previously. The sample was clamped immediately between two large aluminum blocks precooled in liquid nitrogen. The frozen biopsies were divided into three approximately equal transmural sections: epicardium (identified by the methylene blue dye), midmyocardium, and endocardium. The frozen sections were weighed and homogenized at 4°C in 6% perchloric acid using a Tekmar tissue homogenizer. Extracts were neutralized by the procedure outlined previously. Biochemical analyses were performed at 340 nm on a Gilford 250 spectrophotometer. An aliquot of neutralized extract was used in a coupled enzymatic reaction to determine phosphocreatine (PCr) and adenine triphosphate (ATP). In a separate coupled reaction to determine phosphocreatine (PCr) and adenosine monophosphate (AMP) were determined. The tissue nucleotides were expressed as micromoles per gram dry weight. The total adenine nucleotide pool (TAN) was calculated as the sum of ATP, ADP, and AMP content in each layer. Adenylate charge was calculated as (ATP + 1/2 ADP)/TAN.

Tissue slices from normal and postischemic regions were weighed and dried to constant weight at 95°C in prepared vials. Total tissue water (TTW) was expressed as milliliters water per 100 grams dry tissue.

In Vitro Assays

Xanthine oxidase (Sigma Chemical Co., St. Louis, Missouri) was purified by G-100 column chromatography and assayed by measuring the rate of conversion of xanthine to urate as reflected in the absorbance change at 295 nm using a Varian DMS 90 dual beam spectrophotometer. Superoxide generation was determined by measuring the rate of reduction of ferricytochrome c at 550 nm. Peroxidation of xanthine oxidase–treated unsealed erythrocyte membranes (RBC ghosts) was monitored by determining thiobarbituric acid reactive material (MDA) as previously described. The xanthine oxidase–driven lipid peroxidation in RBC ghosts is mediated by the hydroxyl radical.

Human polymorphonuclear leukocytes (PMNs) were obtained from normal volunteers and purified by Ficolle-Hypaque gradient centrifugation followed by hypotonic lysis. PMNs were stimulated with phorbol myristate acetate (PMA) or formyl-methionyl-leucyl-phenylalanine (FMLP), and production of superoxide anion was determined by measuring ferricytochrome c reduction. In the FMLP-stimulated reactions, cytochalasin B (5 μg/ml) was added to the cells during preincubation. Superoxide generation was corrected for a small background basal release. All assay data are expressed as mean values from duplicate samples unless otherwise noted.

Iloprost Pretreatment Protocol

The experimental design included a pretreatment-control measurement of hemodynamics, myocardial segment function, blood flow, and blood gases following instrumentation of the animal (Figure 2). Microscopes were administered before saline or drug intervention. Saline (control series), CON; iloprost at 0.05 μg/kg/min, ILO-LOW; or iloprost at 0.1 μg/kg/min, ILO-HIGH, were infused at the rate of 0.58 ml/min via the right femoral vein 15 minutes prior to LAD occlusion. Ten minutes after drug treatment, hemodynamics and segment function were determined. The LAD was then occluded for 15 minutes, during which hemodynamics, myocardial segment shortening, and myocardial blood flow were determined and blood gas samples obtained. At the end of 15 minutes of occlusion, the infusions were terminated and the occlusion slowly released (over 1 minute) to limit the incidence of severe ventricular arrhythmias and to allow LAD coronary blood flow (CBF) to return to pretreatment control values, i.e., attenuating the reactive hyperemic response. Hemodynamics and myocardial function were determined at 5, 15, 30, 60, 120, and 180 minutes of reperfusion. At 30 and 180 minutes of reperfusion, blood samples for blood gases were obtained and radioactive microspheres were administered to determine regional myocardial blood flow.

FIGURE 2. Experimental protocol used in the stunned myocardium (see "Materials and Methods"). Pretreatment protocol and occlusion-reperfusion protocol are shown. Time of measurements of percent segment shortening and hemodynamics (*) and regional myocardial blood flow and blood samples † are also depicted. PTC, pretreatment-control.
Nitroprusside Pretreatment Protocol

After pretreatment-control measurements, an additional group of dogs was given sodium nitroprusside (NITRO) at a dose (3 μg/kg/min i.v.) that produced nearly equivalent hypotensive effects as ILO-HIGH. The remainder of the protocol was identical to that described for iloprost pretreatment (Figure 2).

Iloprost Occlusion-Reperfusion Protocol

In a final group of dogs, 15 minutes following pretreatment measurements the LAD was occluded, and hemodynamics, myocardial segment shortening, and myocardial blood flow were determined at 12 minutes of occlusion. Immediately following, iloprost (0.1 μg/kg/min i.v.) infusion was begun (ILO-REP group). After 15 minutes of occlusion, the occluder was slowly released allowing CBF to return to preocclusion values. All measurements taken were identical to those described above, and the iloprost infusion was terminated immediately after the 30-minute reperfusion hemodynamic and blood flow measurement.

Statistical Analysis

All values are the mean ± SEM. Hemodynamics were obtained from a mean of 3–5 cardiac cycles. Groups were compared using a two-way analysis of variance with repeated measures design, and Fisher's least significant difference29 was used to test for the significance of difference between any two groups at specific time points isolated from the repeated measures analysis. When occlusion values were compared with the pretreatment control, Dunnett's t test was used. Fisher's exact test30 was used to test for differences in percent ventricular fibrillation between groups. Means were considered significantly different if p<0.05.

Iloprost was kindly supplied by Dr. E. Schillinger (Schering AG, Berlin, FRG) as a 0.5-mg/ml solution in 0.05 M Tris buffer, pH 8.5. A portion of the stock solution was diluted in isotonic saline immediately prior to use. Vehicle infusion had no effect on coronary and systemic hemodynamics.

Results

Of the 52 dogs initially instrumented, 1 (ILO-REP group) died of ventricular fibrillation during ischemia, and 10 died during reperfusion (Figure 3) (5 in the CON group, 2 in the NITRO group, and 3 in the ILO-REP group). When iloprost was administered prior to occlusion (ILO-LOW and ILO-HIGH groups), there were no fibrillations upon reperfusion, and the incidence was significantly less (p<0.02) than that in the control group. The experiment was discontinued in 1 dog (ILO-LOW group) because LAD occlusion failed to produce dyskinesis. Analysis of data was thus performed for 9 control dogs, 7 NITRO-treated dogs, and 8 in each of the 3 iloprost-treatment groups.

Hemodynamics

The hemodynamics of all groups during pretreatment, at 12 minutes of occlusion, and following 3 hours of reperfusion are summarized in Table 1 and Figure 4. All five groups exhibited similar pretreatment values. Nitroprusside and ILO-HIGH produced nearly equivalent reductions in mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), and the heart rate-LVSP product (Figure 4) as compared with their own pretreatment-control values and as compared with the CON group at 10 minutes postdrug and 12 minutes of coronary occlusion (Figure 4). MAP, LVSP, and heart rate-LVSP product were also significantly lower in the ILO-LOW compared with the saline-treated group.

The heart rate-LVSP product remained significantly decreased in the NITRO group at 5 minutes of reperfusion as compared with the CON group (p<0.05). In the ILO-HIGH and ILO-REP groups, the heart rate-LVSP product was significantly decreased at 5 and 15 minutes of reperfusion as compared with the CON group (p<0.05). There were no significant
Table 1. Hemodynamic Data Before, During, and Following Occlusion

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>HR (beats/min)</th>
<th>MAP (mm Hg)</th>
<th>LVSP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>(+) dP/dt (mm Hg/sec)</th>
<th>LAD CBF (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON (9)</td>
<td>151 ± 5</td>
<td>97 ± 6</td>
<td>109 ± 7</td>
<td>3 ± 1</td>
<td>2,650 ± 184</td>
<td>25 ± 4</td>
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<tr>
<td>NITRO (7)</td>
<td>148 ± 7</td>
<td>103 ± 8</td>
<td>113 ± 7</td>
<td>2 ± 1</td>
<td>2,443 ± 221</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>ILO-HIGH (8)</td>
<td>138 ± 6</td>
<td>99 ± 8</td>
<td>115 ± 8</td>
<td>5 ± 1</td>
<td>2,400 ± 197</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>ILO-LOW (8)</td>
<td>149 ± 5</td>
<td>96 ± 5</td>
<td>109 ± 6</td>
<td>3 ± 1</td>
<td>2,494 ± 183</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>ILO-REP (8)</td>
<td>148 ± 6</td>
<td>95 ± 6</td>
<td>104 ± 7</td>
<td>2 ± 1</td>
<td>2,306 ± 171</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Occlusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON (9)</td>
<td>153 ± 5</td>
<td>93 ± 7</td>
<td>104 ± 7</td>
<td>5 ± 1</td>
<td>2,450 ± 194</td>
<td></td>
</tr>
<tr>
<td>NITRO (7)</td>
<td>154 ± 8</td>
<td>74 ± 6†</td>
<td>82 ± 6†</td>
<td>2 ± 1</td>
<td>1,982 ± 214</td>
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</tr>
<tr>
<td>ILO-HIGH (8)</td>
<td>140 ± 5</td>
<td>64 ± 7†</td>
<td>83 ± 7†</td>
<td>4 ± 1</td>
<td>2,035 ± 181</td>
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</tr>
<tr>
<td>ILO-LOW (8)</td>
<td>154 ± 4</td>
<td>80 ± 6†</td>
<td>95 ± 6*</td>
<td>3 ± 1</td>
<td>2,354 ± 179</td>
<td></td>
</tr>
<tr>
<td>ILO-REP (8)</td>
<td>151 ± 6</td>
<td>93 ± 6</td>
<td>103 ± 7</td>
<td>4 ± 1</td>
<td>2,278 ± 159</td>
<td></td>
</tr>
<tr>
<td>3-Hour reperfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON (9)</td>
<td>151 ± 5</td>
<td>104 ± 6</td>
<td>116 ± 6</td>
<td>4 ± 1</td>
<td>2,467 ± 108</td>
<td>16 ± 2</td>
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<tr>
<td>NITRO (7)</td>
<td>146 ± 12</td>
<td>105 ± 8</td>
<td>115 ± 7</td>
<td>3 ± 7</td>
<td>2,186 ± 293</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>ILO-HIGH (8)</td>
<td>140 ± 6</td>
<td>106 ± 8</td>
<td>121 ± 9</td>
<td>5 ± 1</td>
<td>2,269 ± 182</td>
<td>23 ± 4</td>
</tr>
<tr>
<td>ILO-LOW (8)</td>
<td>153 ± 6</td>
<td>101 ± 5</td>
<td>115 ± 5</td>
<td>3 ± 1</td>
<td>2,439 ± 153</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>ILO-REP (8)</td>
<td>141 ± 6</td>
<td>102 ± 6</td>
<td>110 ± 6</td>
<td>3 ± 1</td>
<td>2,260 ± 139</td>
<td>24 ± 3</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Numbers in parentheses are the number of dogs in each group. HR, heart rate; MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LAD CBF, left anterior descending coronary blood flow; CON, saline-treated; NITRO, nitroprusside; ILO-HIGH, iloprost at 0.1 µg/kg/min; ILO-LOW, iloprost at 0.05 µg/kg/min; ILO-REP, iloprost at 0.1 µg/kg/min 2 minutes prior to reperfusion. Significant differences as compared with the control (CON) group are indicated by *p<0.05 and †p<0.01.

Table 2. Ischemic Bed Size and Regional Myocardial Blood Flow

<table>
<thead>
<tr>
<th></th>
<th>LV wt (g)</th>
<th>Area at risk wt (g)</th>
<th>%LV at risk</th>
<th>Transmural collateral blood flow (ml/min/g)</th>
<th>Ischemic area endo/epi</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON (9)</td>
<td>86.4 ± 4.0*</td>
<td>24.0 ± 1.6</td>
<td>27.8 ± 1.3</td>
<td>0.17 ± 0.04</td>
<td>0.61 ± 0.08</td>
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<tr>
<td>NITRO (7)</td>
<td>93.0 ± 8.1</td>
<td>28.9 ± 2.7</td>
<td>31.8 ± 2.8</td>
<td>0.14 ± 0.04</td>
<td>0.71 ± 0.11</td>
</tr>
<tr>
<td>ILO-HIGH (8)</td>
<td>99.7 ± 10.6</td>
<td>28.1 ± 2.6</td>
<td>29.1 ± 3.0</td>
<td>0.15 ± 0.05</td>
<td>0.75 ± 0.09</td>
</tr>
<tr>
<td>ILO-LOW (8)</td>
<td>100.3 ± 6.3</td>
<td>30.4 ± 2.5</td>
<td>30.5 ± 2.1</td>
<td>0.17 ± 0.05</td>
<td>0.53 ± 0.07</td>
</tr>
<tr>
<td>ILO-REP (8)</td>
<td>90.8 ± 5.3</td>
<td>28.7 ± 2.1</td>
<td>31.4 ± 1.3</td>
<td>0.17 ± 0.02</td>
<td>0.53 ± 0.07</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM. Numbers in parentheses are the number of dogs in each group. Drug abbreviations are similar to those in Table 1. LV, left ventricle; endo/epi, endocardial-to-epicardial blood flow ratio.
Myocardial segment function (calculated as %SS) of the LAD region prior to drug treatment was similar in the CON, ILO-LOW, and ILO-HIGH groups (Figure 5). Infusion of iloprost or saline did not significantly influence %SS during the initial 15 minutes prior to LAD occlusion. In the ischemic-reperfused LAD region, coronary occlusion resulted in a marked reduction in %SS to negative values. The decrease in %SS was not different between the three groups (CON group, %SS = -7.4 ± 1.9; ILO-HIGH group, %SS = -6.6 ± 2.0; ILO-LOW group, %SS = -6.3 ± 2.0), which suggests that iloprost treatment did not alter the severity of ischemia during occlusion.

Functional recovery of the ischemic-reperfused region of the three groups during reperfusion is illustrated in Figure 5. In the CON group, %SS was depressed throughout 3 hours of reperfusion as compared with the preocclusion period. At the end of reperfusion, %SS had only returned to 3% of the pretreatment level.

In the ILO-LOW group, recovery of %SS of the ischemic-reperfused region was significantly greater than that of the CON group with the exception of 15 minutes (Figure 5). Recovery of function as compared with the CON group was maximal at 2 and 3 hours of reperfusion (p<0.01).

In the ILO-HIGH group, %SS recovered to 81% of the pretreatment level at 5 minutes of reperfusion (Figure 5). The recovery of segment function was significantly greater than that of the CON group (p<0.001) throughout reperfusion and was greater than that of the ILO-LOW group (p<0.05) from 5 minutes through 1 hour of reperfusion after which there was no significant difference in recovery between the two iloprost-treated groups.

Nitroprusside Pretreatment Protocol

In the ischemic-reperfused region (Figure 6), nitroprusside-treated dogs showed a similar decrease in %SS during coronary occlusion to that observed in the ILO-HIGH group. The recovery of %SS of the NITRO group was significantly greater (p<0.001) than that of the CON group only at 5 minutes of reperfusion. At 3 hours of reperfusion, %SS in the NTTRO group was 19% of pretreatment-control values.

Recovery of %SS in the ILO-HIGH group was significantly greater than that of the CON group throughout reperfusion (p<0.001) and of that observed in the NITRO group at 15 (p<0.05), 30, 60, 120, and 180 minutes (p<0.01) of reperfusion. The ILO-LOW group also had an enhanced recovery of %SS as compared with the NITRO group at 60, 120, and 180 minutes of reperfusion (p<0.05).

Iloprost Occlusion-Reperfusion Protocol

In the ILO-REP group, coronary occlusion resulted in similar decreases in %SS as compared with the CON group (Figure 7). When iloprost (0.1 µg/kg/min i.v.) was given near the end of occlusion and throughout 31 minutes of reperfusion, the recovery in %SS was significantly greater when compared with the CON group at all periods of reperfusion (p<0.05). However,
Myocardial Metabolism

The ATP, PCr, TAN, and TTW values of the ischemic and nonischemic region in all groups are summarized in Tables 3 and 4. By 3 hours of reperfusion, ATP in the ischemic-reperfused region was significantly less (p<0.05) than that in the nonischemic region in all myocardial layers of CON, ILO-LOW, NITRO, and ILO-REP groups and less in the endocardium and midmyocardium in the ILO-HIGH group (Table 3). There were no differences in absolute values of ATP between groups. A rebound of PCr was seen in the endocardial and midmyocardial layers in all groups except the NITRO group where PCr was significantly elevated only in the endocardium (Table 3). With the exception of the epicardium in the ILO-HIGH group, total adenine nucleotides in all groups were always lower in the ischemic-reperfused region than in the corresponding layers of the normal region (Table 3). There were no differences between the groups in adenylyl charge in the normal or reperfused regions (range between groups = 0.90–0.92).

Table 4 shows TTW in ischemic and nonischemic areas. While there was an increase in TTW in all layers of the ischemic-reperfused region, statistically significant increases occurred predominantly in the endocardium. Drug treatment did not significantly influence ischemia-induced increases in TTW.

In Vitro Assays

The effects of iloprost on the xanthine oxidase free-radical generating system in vitro are shown in Table 5. Iloprost had no influence on the enzyme xanthine oxidase as demonstrated by urate production and exhibited no superoxide scavenging action as indicated by the lack of effect on superoxide dismutase inhibitable superoxide production (ferricytochrome c reduction). Lipid peroxidation, which was completely inhibited by superoxide dismutase or catalase, was unaffected by iloprost treatment.

Iloprost had no effect on superoxide production by PMA-stimulated PMNs (Table 5). In contrast, iloprost inhibited, in a concentration-dependent manner, the superoxide burst by PMNs stimulated with the chemotactic agent FMLP (Figure 8). The concentration of iloprost that produced half-maximal inhibition was 0.65 μg/ml (1.8 μM).

Discussion

One major finding in the present study is that the prostacyclin analogue, iloprost, produced a dose-dependent increase in the recovery of regional myocardial segment function following 15 minutes of coronary artery occlusion and 3 hours of reperfusion and that this beneficial effect was greater when iloprost was administered prior to occlusion versus immediately prior to reperfusion. Second, functional recovery was enhanced in all three iloprost-treated groups despite a lack of improvement in collateral blood flow, endo/epi blood flow ratio, tissue high energy phosphates, and hemodynamics during reperfusion. Finally, results obtained in vitro indicate that iloprost inhibits the superoxide burst induced by stimulation of neutrophils with the chemotactic agent FMLP, and these findings suggest that part of the beneficial action of iloprost to enhance functional recovery in the stunned myocardium may be mediated by an oxygen free-radical scavenging mechanism.

Role of Hemodynamics in the Effect of Iloprost

The control group showed minimal recovery in regional function (calculated as %SS) throughout 3 hours of reperfusion. This is consistent with previously reported results.16,31 We have also shown that administration of ILO-HIGH, which produced the greatest effect on recovery, resulted in the largest decrease in MAP and the heart rate-LVSP product during the occlusion and early reperfusion period. Despite this seemingly beneficial effect on hemodynamics, hypotension per se appears to play only a minor role in the recovery of the stunned myocardium because an equihypotensive dose of sodium nitroprusside showed no sustained improvement in function during reperfusion. This finding is in agreement with that of Przyklenk and Kloner,31 who found no beneficial effect on functional recovery after administration of nitroprussi-
Iloprost and Reperfusion Injury

In their study, however, nitroprusside was given during the reperfusion period and would not be an adequate control for agents that significantly affect hemodynamics prior to and during the occlusion period. In other models, iloprost has been shown to elicit beneficial effects independent of hemodynamic mechanisms. Iloprost was found to prolong exercise duration and time to onset of ST-segment depression in patients with effort-induced angina. These improvements were independent of changes in myocardial oxygen demand and were postulated to be associated with a reduction in platelet aggregation. However, Szekeres et al found that the greatest protective effect of iloprost to prevent or reduce ST-segment elevation appeared at a time when blood pressure and platelet aggregation had returned to normal. Thus, the results of the present study and those of others indicate that the hypotensive effect of iloprost cannot explain its beneficial effect on functional recovery.

**Antifibrillatory Effects of Iloprost**

Iloprost has been reported to be antiarrhythmic or arrhythmogenic depending on the concentrations used, routes of administration, and the species of animal studied. Coker and Parratt found an increased incidence of tachyarrhythmias in the presence of iloprost after a longer occlusion period (40 minutes) than the one used in the present study. This only occurred when iloprost caused a reflex increase in heart rate, an effect not observed in our study. In contrast, we found that iloprost administration prior to coronary artery occlusion reduced (p<0.02) the incidence of ventricular fibrillation upon reperfusion but not the incidence of arrhythmias during occlusion. This antifibrillatory effect was not due to its hemodynamic actions since this effect was not mimicked by nitroprusside. This observation is consistent with findings of Fiedler and Mardin who found that PGI2 administered intravenously reduced the incidence of ventricular fibrillation.

### Table 3. High Energy Phosphates at 3 Hours of Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Ischemic region</th>
<th>Nonischemic region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epicardium</td>
<td>Mid</td>
</tr>
<tr>
<td>ATP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>19.5 ± 1.0†</td>
<td>19.3 ± 0.5†</td>
</tr>
<tr>
<td>NITRO</td>
<td>20.5 ± 1.3†</td>
<td>17.2 ± 1.1†</td>
</tr>
<tr>
<td>ILO-HIGH</td>
<td>22.5 ± 1.4</td>
<td>20.0 ± 1.3†</td>
</tr>
<tr>
<td>ILO-LOW</td>
<td>18.0 ± 1.4*</td>
<td>17.0 ± 1.4†</td>
</tr>
<tr>
<td>ILO-REP</td>
<td>19.6 ± 1.1†</td>
<td>17.5 ± 1.0†</td>
</tr>
<tr>
<td>PCr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>51.8 ± 5.1</td>
<td>61.9 ± 4.5†</td>
</tr>
<tr>
<td>NITRO</td>
<td>52.9 ± 4.7</td>
<td>49.5 ± 4.9</td>
</tr>
<tr>
<td>ILO-HIGH</td>
<td>52.2 ± 3.1</td>
<td>56.6 ± 3.5*</td>
</tr>
<tr>
<td>ILO-LOW</td>
<td>37.9 ± 1.6</td>
<td>48.6 ± 2.8†</td>
</tr>
<tr>
<td>ILO-REP</td>
<td>46.3 ± 3.2</td>
<td>47.2 ± 2.9†</td>
</tr>
<tr>
<td>Total Adenine Nucleotides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>24.6 ± 1.3†</td>
<td>23.2 ± 0.6†</td>
</tr>
<tr>
<td>NITRO</td>
<td>24.3 ± 1.4*</td>
<td>20.6 ± 1.2†</td>
</tr>
<tr>
<td>ILO-HIGH</td>
<td>26.2 ± 1.6</td>
<td>23.4 ± 1.5†</td>
</tr>
<tr>
<td>ILO-LOW</td>
<td>21.4 ± 1.9*</td>
<td>20.2 ± 1.6†</td>
</tr>
<tr>
<td>ILO-REP</td>
<td>23.3 ± 1.2†</td>
<td>21.4 ± 1.0†</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. Tissue ATP, phosphocreatine (PCr), and total adenine nucleotides are expressed in units of micromoles per gram dry weight. Drug abbreviations are similar to those found in Table 1. Significant differences between ischemic and nonischemic regions within a single layer in the same group are indicated by *p<0.05 and †p<0.01.

### Table 4. Total Tissue Water at 3 Hours of Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Ischemic region</th>
<th>Nonischemic region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epicardium</td>
<td>Mid</td>
</tr>
<tr>
<td>CON</td>
<td>381.3 ± 13.5</td>
<td>384.3 ± 8.5</td>
</tr>
<tr>
<td>NITRO</td>
<td>398.1 ± 8.2†</td>
<td>379.8 ± 17.2*</td>
</tr>
<tr>
<td>ILO-HIGH</td>
<td>356.9 ± 7.5</td>
<td>373.4 ± 10.2†</td>
</tr>
<tr>
<td>ILO-LOW</td>
<td>364.1 ± 13.1*</td>
<td>376.6 ± 12.5*</td>
</tr>
<tr>
<td>ILO-REP</td>
<td>374.2 ± 12.6</td>
<td>361.0 ± 13.3*</td>
</tr>
<tr>
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<td></td>
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<td></td>
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</tbody>
</table>

Values are the mean ± SEM (ml H2O/100 g dry wt). Drug abbreviations are similar to those in Table 1. Significant differences between ischemic and nonischemic regions within a single layer in the same group are indicated by *p<0.05 and †p<0.01.
Table 5. Effect of Iloprost on Xanthine Oxidase and PMN Free-Radical Generating Systems

<table>
<thead>
<tr>
<th></th>
<th>Urate (nmol/min)</th>
<th>O₂ (nmol/min)</th>
<th>Lipid peroxidation (nmol MDA/mg protein)</th>
<th>O₂ (nmol/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.6 ± 0.2*</td>
<td>11.0 ± 0.3†</td>
<td>7.1 ± 0.14†</td>
<td>10.1 ± 0.5§</td>
</tr>
<tr>
<td>+ SOD (50 μg/ml)</td>
<td>...</td>
<td>0.8</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>+ CAT (50 μg/ml)</td>
<td>...</td>
<td>0.3</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>+ ILO (0.01 μg/ml)</td>
<td>5.9</td>
<td>10.7</td>
<td>7.2</td>
<td>9.6</td>
</tr>
<tr>
<td>+ ILO (1 μg/ml)</td>
<td>5.8</td>
<td>11.2</td>
<td>7.3</td>
<td>9.6</td>
</tr>
<tr>
<td>+ ILO (10 μg/ml)</td>
<td>5.6</td>
<td>10.9</td>
<td>7.5</td>
<td>9.9</td>
</tr>
<tr>
<td>+ ILO (100 μg/ml)</td>
<td>...</td>
<td>0.7</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>+ SOD (50 μg/ml)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Control conditions were *8 μg/ml xanthine oxidase, 0.1 mM xanthine, 0.1 mM EDTA in 50 mM sodium phosphate (pH 7.8); †16 μg/ml xanthine oxidase, 0.1 mM xanthine, 8 μM ferricytochrome c, 0.1 mM EDTA in 50 mM sodium phosphate (pH 7.8); ‡10 μg/ml xanthine oxidase, 1 mM xanthine, 100 μM FeCl₃, mg protein/ml RBC ghosts in phosphate-buffered saline (pH 7.4) (60-minute reaction), and §1 x 10⁶ PMNs, 30 μM ferricytochrome c, 100 ng/ml PMA (0.14 M DMSO) in Hank’s buffered salt solution (5-minute reaction). Numbers with ± designations are mean ± SD values from duplicate experiments.

The finding is in agreement with previous work of Smith et al. However, it appears to conflict with that of Jugdutt et al. who found that PGI₂ infusion reduced infarct size and increased collateral blood flow in dogs with a permanent coronary artery occlusion. Differences between the results of Jugdutt et al. and those of the present study on collateral perfusion are not apparent but may be related to the use of iloprost rather than PGI₂, the model of ischemia, or differences in hemodynamics. In addition, a measurement of collateral flow before and after iloprost infusion was not performed in the present study. Therefore, it is difficult to evaluate the effect of the drug on collateral perfusion. Nevertheless, it is unlikely that the cardioprotective effect of iloprost is due to a drug-induced increase in coronary collateral flow.

Effects of Iloprost on Collateral Blood Flow

Iloprost-treated animals showed no differences in coronary collateral blood flow during occlusion of the LAD as compared to the CON and NITRO groups. This finding is in agreement with previous work of Smith et al. It also appears to conflict with that of Jugdutt et al. who found that PGI₂ infusion reduced infarct size and increased collateral blood flow in dogs with a permanent coronary artery occlusion. Differences between the results of Jugdutt et al. and those of the present study on collateral perfusion are not apparent but may be related to the use of iloprost rather than PGI₂, the model of ischemia, or differences in hemodynamics. In addition, a measurement of collateral flow before and after iloprost infusion was not performed in the present study. Therefore, it is difficult to evaluate the effect of the drug on collateral perfusion. Nevertheless, it is unlikely that the cardioprotective effect of iloprost is due to a drug-induced increase in coronary collateral flow.

Time Dependency of Iloprost

When iloprost was administered immediately prior to reperfusion, there was a significant improvement in regional function compared with the CON group. Thus, some of the beneficial effects of iloprost appear to occur during the reperfusion period. Functional recovery was also significantly greater when giving an equivalent dose of iloprost during preocclusion than just prior to reperfusion. This suggests that iloprost has beneficial actions before and/or during occlusion or that the blood level of iloprost was not high enough to be of maximal benefit when given late in occlusion.

Role of High Energy Phosphates

The results of the present experiments agree with those of van der Giessen and colleagues and indicate that iloprost has no effect on high energy phosphate content during reperfusion despite the enhanced recovery of function. Therefore, postischemic improvement in myocardial function is not apparently related to an iloprost-induced increase in myocardial high energy phosphates. Recently, there have been other studies suggesting a poor correlation between recovery of ATP and myocardial function during reperfusion.
Becker and coworkers have recently shown that postischemic dysfunction in the stunned myocardium may be reversed by epinephrine and postextrasystolic potentiation. In addition, Arnold et al. showed that the administration of another positive inotropic agent, dopamine, during reperfusion improved contractile performance despite reduced ATP concentrations. Thus, the problem in the stunned myocardium may well be one of uncoupling of contraction with usage of ATP.

**Cytoprotective Actions of Iloprost**

Total tissue water data showed that iloprost-treated groups had lower myocardial tissue water in the ischemic-reperfused region than the control or nitroprusside-treated groups. However, TTW was also lower in the normal region in the iloprost-treated animals. The reason(s) for these differences is not clear nor is the effect that they would subsequently have on functional recovery. However, we cannot exclude the possibility that the reduction in TTW produced by iloprost is mediated through a cytoprotective action on membrane permeability as part of its mechanism of action in improving function. In support of a cytoprotective effect, it has been shown that either prostacyclin or iloprost preserves membrane integrity in the ischemic rabbit, cat heart, hypoxic cat liver, and suspensions of liver lysosomes, and that these effects were unrelated to their antiaggregatory or vasodilatory actions.

**Effect of Oxygen Free Radicals in the Beneficial Actions of Iloprost**

Much evidence has accumulated showing that there is a release of oxygen-derived free radicals during ischemia and reperfusion that may lead to lipid peroxidation and membrane damage. With the use of oxygen free-radical scavengers, recovery of myocardial function in the stunned myocardium has been shown to be enhanced. Thiemermann et al. found that iloprost prevented an ischemia-induced decrease in superoxide dismutase activity. It was difficult to determine from this study whether superoxide dismutase preservation was due to a direct action of iloprost on free-radical generating systems, some type of scavenging effect of the drug, or simply an effect on the severity of ischemia, resulting in a reduced loss of superoxide dismutase activity. In the present study, iloprost had no effect in vitro on the xanthine oxidase system that has been proposed to mediate reperfusion injury via free-radical formation. In addition, iloprost had no effect on xanthine oxidase-induced lipid peroxidation in RBC ghosts. We have, however, demonstrated that iloprost reduces the in vivo neutrophil-derived superoxide burst after stimulation with the chemotactic peptide FMLP but not after PMA. These findings are in agreement with those of Fantone and Kinnes and Simpson et al., who also demonstrated similar effects with PGI2, on neutrophil superoxide production induced by FMLP and observed both prostaglandin-dependent and -independent pathways for regulating superoxide anion production in PMNs. These findings also suggest that iloprost acts some-

where in the pathway of signal transduction between receptor (FMLP) and protein kinase C by PMA, possibly at the G protein level. Stimulation of PMNs by a chemotactic agent probably represents a more pathophysiologic situation as compared with direct activation of protein kinase C by PMA.

Fantone and Kinnes found that the concentration of PGI2, that produced a 50% inhibition of neutrophil superoxide production in vitro was 30 μM. Simpson et al demonstrated that an infusion of 50 ng/kg/min of PGI2 to dogs reduced myocardial infarct size and inhibited neutrophil migration in vivo. Based on these results, Simpson et al. concluded that the mechanism of action of PGI2 in reducing infarct size in dogs is via a reduction in neutrophil activation. The present results indicate that iloprost, an agent similar in potency to PGI2, inhibits neutrophil activation 50% in vitro at 1.8 μM, a concentration 17 times lower than that observed in a similar study in canine neutrophils with PGI2, and improves postischemic function in vivo following infusions of 50 and 100 ng/kg/min. Thus, although speculative, the present results suggest that at least part of the mechanism of action of iloprost in improving postischemic dysfunction in the stunned myocardium may be the result of its inhibitory effect on neutrophil function. On the other hand, it is not certain as to the importance of neutrophils or neutrophil-derived free radicals on postischemic dysfunction observed in the stunned myocardium; however, previous results from this laboratory and others suggest a probable role for oxygen-derived free radicals in mediating the damage seen in this model. Also, neutrophils have been shown to be important as mediators of damage in models involving longer occlusion periods.

In conclusion, the prostacyclin-mimetic, iloprost, ameliorates postischemic dysfunction in the stunned myocardium in a dose- and time-dependent manner. These effects do not appear to be mediated by either changes in classic oxygen supply-demand mechanisms such as collateral blood flow or the heart rate-LVSP product or preservation of myocardial adenine nucleotides. Instead, our in vitro experiments suggest that the mechanism by which iloprost improves recovery of myocardial function may be mediated via inhibition of oxygen-derived free radicals.

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Key Words • myocardial ischemia • iloprost • regional myocardial function • coronary reperfusion • sodium nitroprusside • neutrophil superoxide production
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