Role of Prostacyclin in the Preservation of Ischemic Myocardial Tissue in the Perfused Cat Heart

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SUMMARY Prostacyclin (PGI₂) and some of its major breakdown products (6-keto-PGF₁α, 6-keto-PGE₁α, and 13,14-dihydro 15-keto-PGF₁α) were studied in an isolated perfused cat heart preparation during myocardial ischemia. At an infusion rate of 10 ng/g heart weight per minute, PGI₂ and the related compounds caused no changes in perfusion pressure, contractile force (CF), the first derivative of the contractile force (dF/dt), and heart rate in control hearts perfused at coronary flows of 20–35 ml/min. Induction of global ischemia by perfusion at 0.6 to 0.7 ml/min for 120 minutes resulted in a significant release of creatine kinase (CK) activity and compounds having a free amino-nitrogen group into the perfusate. Ischemic hearts exhibited an increase in resting tension of 2.3 ± 0.2 g, mean ± SEM. Upon reperfusion, untreated ischemic hearts showed a partial restoration of mechanical performance, CF = 43 ± 5%, and dF/dt = 40 ± 5% of control. PGI₂ infusion inhibited the ischemic-induced CK release and the increase in perfusate amino-nitrogen concentration. Resting tension also remained low (i.e., 0.8 ± 0.1 g). Recovery of CF and dF/dt upon reperfusion was significantly higher (86 ± 8% and 88 ± 10%, respectively) than in the untreated ischemia group. Myocardial CK activity was significantly higher in PGI₂-infused hearts (35.8 ± 2.6 IU/mg protein) compared to those infused with its vehicle (26.3 ± 2.8, P < 0.01). Breakdown products of PGI₂ only slightly protected against ischemia. PGI₂ is beneficial in myocardial ischemia in vitro, even without its well known action preventing platelet aggregation and independent of its induction of coronary vasodilation. Circ Res 47: 757-763, 1980

PROSTACYCLIN (PGI₂) is produced primarily by the endothelial cells of blood vessels and is a potent inhibitor of platelet aggregation (Moncada et al., 1976), thus potentially playing an important role in maintaining flow within blood vessels. PGI₂ is also a potent vasodilator in many organs including the heart (Dusting et al., 1977; Ogletree et al., 1978). Recently, PGI₂ has been shown to be beneficial in circulatory shock (Lefer et al., 1979), myocardial ischemia (Ogletree et al., 1979), cerebral ischemia (Hallenbeck and Furlow, 1979), and in animals and patients in arteriosclerosis obliterans (Szzczeklik et al., 1979). We have reported previously a cytoprotective effect of PGI₂ during hypoxic perfusion of the liver independently of its effects on platelet aggregation and vascular tone (Araki and Lefer, 1980a). The major protective mechanism in the hypoxic liver appears to be preservation of cell integrity. Others have reported the cytoprotective effect of PGI₂ on gastric mucosal lesions produced by ethanol (Robert et al., 1978). Thus it is important to examine the direct effect of PGI₂ on ischemic myocardial injury using a constant flow controlled isolated heart perfused without blood cells to eliminate the actions of PGI₂ on the coronary vasculature and on platelet aggregation. This study was performed to clarify the role of PGI₂ as well as some of its breakdown products in preserving cell integrity during myocardial ischemia and to clarify the mechanisms of any potential beneficial effect.

Methods

Cats of either sex weighing 2.6–3.3 kg were anesthetized by intravenous administration of sodium pentobarbital (30 mg/kg), and were given heparin (500 U/kg) intravenously. The heart was excised rapidly, placed in Krebs-Henseleit solution, and mounted in a siliconized glass Langendorff perfusion apparatus. The heart was perfused retrogradely through the aorta with 250 ml of Krebs-Henseleit solution, and mounted in a siliconized glass Langendorff perfusion apparatus. The heart was perfused retrogradely through the aorta with 250 ml of Krebs-Henseleit solution titrated to pH 7.3. Oxygen tension of the perfusate was maintained at 475–500 mm Hg by bubbling a gas mixture of 95% O₂ + 5% CO₂ through the perfusate within the reservoir. A 30-minute equilibration period was employed before recirculation of the perfusate to the heart to allow all blood to be flushed out of the system. Recirculation of the perfusate was then maintained for the remainder of the experiment. The flow rate of the pump was adjusted to yield a coronary perfusion pressure of 50–60 mm Hg. This provided a fixed flow rate of 20–35 ml/min in each heart except during the ischemic period. A Haake constant-temperature circulator was used to maintain perfusate temperature at 37.0 ± 0.2°C at the inflow to the

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heart. Coronary perfusion pressure was measured by a Statham P23Db pressure transducer through a cannula positioned at the inflow tubing. Surgical silk was sutured to the apex of heart and connected to a force displacement transducer (Grass FT03C), through a pulley, thus measuring the contractile force of the heart. Diastolic tension was set at 0.5 g initially. The first derivative of contractile force (dF/dt) was measured by a differentiating coupler (Beckman, Type 9879), and all variables were displayed on an oscillographic ink-writing recorder (Beckman, type R411). Heart rate was measured by counting the contractions for 60 seconds at a paper speed of 10 mm/sec. Ischemia was induced by reducing the coronary flow to 0.6-0.7 ml/min at a perfusion pressure of 15-20 mm Hg. Two hours after the onset of ischemia, each heart was reperfused at the pre-ischemic flow rate for an additional 30 minutes. The coronary effluent was sampled each 30 minutes and used for the measurement of creatine kinase (CK) activity and amino-nitrogen concentration. At the end of each experiment, transmural tissue samples were taken from the free wall of the left ventricle and used for the measurement of tissue CK activity and water content.

Analytical Procedures
Perfusate Po2 was determined every 30 minutes by use of a blood gas analyzer (Instrumentation Labs., model 313). At the end of each experiment, approximately 0.2 g of cardiac tissue sample was minced, suspended in a solution of cold 0.25 M sucrose, containing 1 mM EDTA and 0.1 mM mercaptoethanol (1:10, wt/vol), and homogenized by a Polytron (Brinkman, PCU-2) homogenizer. Homogenates were centrifuged at 36,000 g for 30 minutes, and the supernatant was used for the measurement of CK activity and protein concentration. Protein concentration was measured by the biuret method. CK activity was measured by the method of Rosalki (1967) at 25°C and expressed as IU/ml for perfusate or IU/mg protein for tissue. Perfusate amino-nitrogen concentrations were measured using the ninhydrin method (Kabat, 1961) and expressed as umoles serine/ml of perfusate. To determine the water content of the tissue, about 1 g of heart sample was blotted carefully on filter paper, wet weight measured, and the tissue sample desiccated in a drying oven at 60°C for 2-3 days until the tissue weight became constant. Water content was expressed as ml/g dry tissue.

Drugs
PGI2 and some of its metabolites and breakdown products; 6-keto-prostaglandin F1α (6-keto-PGF1α), 6-keto-prostaglandin E1 (6-keto-PGE1), 13,14-dihydro 6,15-diketo-prostaglandin F1α (13,14-dihydro 6,15-diketo PGF1α) were dissolved in 100% ethanol until use. Before each experiment, PGI2 was diluted to a suitable concentration with 1 mM Tris buffered to pH 10.0, and its metabolites were diluted with 1 mM Tris solution at pH 8.0. Each drug was infused at a rate of 10 ng/g heart weight per minute starting 15 minutes prior to ischemia and continuing throughout the experiment. Heart weight ranged from 12 to 19 g. The vehicle for PGI2 (i.e., 1 mM Tris at pH 10.0) was infused at the same rate of 0.3 ml/hr as PGI2. The vehicle at both pH 8 and 10 was found to exert no detectable effect on cardiodynamic or biochemical measurements.

Statistics
All values described in the text, figures, and tables are means ± SEM. Significance of results were determined using linear regression analysis and confirmed by Student's t-test for unpaired data. P values of less than 0.05 were considered statistically significant.

Results
Figures 1, 2, and 3 show representative tracing from three experimental groups. As shown in Figure 1, infusion of PGI2 at a rate of 10 ng/g heart weight per minute induced no significant change in the perfusion pressure, contractile force, dF/dt, and heart rate. At higher infusion rates (i.e., 30 ng/g heart weight per minute), a significant drop in coronary perfusion pressure was observed (data not shown). When normoxic hearts were perfused with PGI2, none of the variables changed significantly throughout the experiment (Fig. 1). However, in the ischemic groups, reduction of coronary flow induced a transient increase in contractile force and dF/dt in the presence of either PGI2 or its vehicle, followed by cardiac arrest in diastole within 2 minutes in both situations (Figs. 2 and 3). Upon reperfusion, the heart receiving the vehicle showed a...
poor recovery, whereas the PGI2-treated heart (bottom panel) recovered almost completely 30 minutes after reperfusion. Since all recordings reached a stable level 30 minutes after reperfusion, hemodynamic values were expressed as percent recovery from initial values. Table 1 summarizes the percent recovery of perfusion pressure (i.e., coronary resistance at constant flow), contractile force, dF/dt, and heart rate. Coronary vascular resistance and heart rate completely recovered in ischemic hearts given either vehicle or PGI2. However, the recovery of contractile force and dF/dt was significantly higher in the PGI2-treated ischemic group than in ischemic hearts receiving only the vehicle. Moreover, the resting tension increased markedly during ischemia in hearts receiving the vehicle. In contrast, PGI2 infusion inhibited the rise of resting tension observed during ischemia. A significant difference was observed in resting tension at 120 minutes between ischemic hearts perfused with PGI2 or vehicle (P < 0.001, Fig. 4).

Figure 5 represents changes in the perfusate CK activity in three experimental groups of perfused hearts. The control group given PGI2 showed no increase in the perfusate CK activity during the experiment. However, ischemic hearts given only the vehicle showed significant increases in circulating CK activity compared to the control group (P < 0.01 at 60 minutes). PGI2 infusion significantly inhibited CK release into the perfusate. This inhibition of CK release was confirmed by the measurement of tissue CK activity at the end of the experiment. As shown in Figure 6, ischemic hearts perfused with vehicle experienced a decrease in tissue CK activity compared to the control hearts. However, ischemic perfused hearts given PGI2 showed a significant retention of tissue CK activity (P < 0.01 compared to ischemia + vehicle group).

Figure 7 illustrates the changes in perfusate amino-nitrogen concentrations during the first 30 and 60 minutes of perfusion. PGI2 infusion significantly inhibited the increase in the perfusate amino-nitrogen concentration compared to its vehicle at both times. Significant differences between the two groups were also observed up to 90 minutes after ischemia. However, at 120 minutes, no significant difference was observed, since the concentration of amino-nitrogen increased in PGI2 treated ischemic hearts at 120 minutes. Thus, PGI2 only retarded the rate of accumulation of amino-nitrogen during ischemia.

Tissue water content increased significantly in ischemic perfused hearts given only the vehicle (5.31 ± 0.19 ml/g dry weight) compared to control hearts (4.73 ± 0.10, P < 0.025). However, ischemic perfused hearts given PGI2 showed similar water contents (4.80 ± 0.12) as control perfused hearts, indicating a prevention of edema formation by PGI2.

Table 2 summarizes the effect of the major PGI2 metabolites and the vehicle during ischemia, as well as the effect of the vehicle on control perfused hearts. Infusion of the vehicle during ischemia resulted in large changes in all variables measured, indicating no significant effect of the vehicle during ischemic perfusion. Similarly, 6-keto PGF1α showed no protective effect on any of the variables examined. However, 6-keto-PGE1 and 13,14-dihydro 6,15 diketo PGF1α showed a partial protective effect during ischemia; 6-keto PGE1 inhibited the ischemia-induced increases in perfusate CK activity, and tissue water content, whereas 13,14-dihydro 6,15-diketo PGF1α showed a significantly higher recovery of contractile force and dF/dt, and a lower resting tension and tissue water content compared to its vehicle. Thus, some PGI2 metabolites exert a few protective effects of their parent compounds.

**Discussion**

In the present study, significant improvement of cardiac function and moderation of ischemic depletion of tissue CK activity by PGI2 were observed.
TABLE 1  Percent Recovery of Hemodynamic Variables after Reperfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Contractile force</th>
<th>dF/dt</th>
<th>Coronary vascular resistance</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + PGI₂</td>
<td>8</td>
<td>104 ± 6</td>
<td>104 ± 11</td>
<td>86 ± 4</td>
<td>97 ± 4</td>
</tr>
<tr>
<td>Ischemia + vehicle</td>
<td>10</td>
<td>43 ± 5</td>
<td>40 ± 5</td>
<td>98 ± 7</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>Ischemia + PGI₂</td>
<td>9</td>
<td>86 ± 8*</td>
<td>88 ± 10*</td>
<td>81 ± 6</td>
<td>92 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SEM expressed as percent of zero time value. n = number of hearts perfused in each group.

* P < 0.001 compared to Ischemia + vehicle.

upon reperfusion following 120 minutes of ischemia. Since the perfusate included no platelets, the beneficial action of PGI₂ in the isolated perfused cat heart does not appear to depend on prostacyclin's inhibitory effect on platelet aggregation, which occurs in myocardial ischemia in vivo (Vik-Mo, 1978; Ogletree et al., 1979; Leinberger et al., 1979). In addition, total coronary flow was controlled by a constant flow pump. During reperfusion, coronary vascular resistance did not differ between the ischemic heart given PGI₂ and that given the vehicle. Thus, it seems unlikely that enhancement of tissue perfusion is a major factor in the recovery observed following PGI₂ infusion. Of course, we cannot completely exclude the possibility of effects of PGI₂ on shunt flow or distribution of myocardial flow, or on the microcirculation. Whatever the precise mechanism of action of PGI₂, its beneficial effect is manifested during the ischemic period, as evidenced by retarding CK and aminonitrogen release and by lower resting tension. Furthermore, the total coronary flow is so low (e.g., 0.6-0.7 ml/min) during the ischemic period that any potential effect of PGI₂ on nutritive flow would unlikely be of sufficient magnitude to account for the protective effect. Furthermore, PGI₂ does not improve the transmural distribution of coronary flow during ischemia (i.e., endocardial to epicardial flow ratio) (Lefer and Smith, 1979; Jentzer et al., 1979). Thus, the beneficial effect of prostacyclin in postmyocardial ischemia reperfusion is independent of its antiaggregation effect in platelets and very likely independent of its vasodilator action.

PGI₂ also showed no significant effect on contractile force, dF/dt, and heart rate at the dose employed, confirming the previous report of a lack of inotropic effect on papillary muscles (Lefer et al., 1978) and in intact dogs (Jentzer et al., 1979). The increase in contractile force and dF/dt just after the onset of ischemia was comparable in hearts given PGI₂ or its vehicle. Furthermore, the interval between the induction of ischemia and cardiac arrest was similar between the two ischemia groups; hearts from both groups uniformly arrested (i.e., standstill) throughout the entire ischemic period.
Thus, the protective effect of PGI$_2$ on ischemia seems not to be due to any effect on cardiac work or to modifying myocardial oxygen demand.

The precise molecular mechanism of the cyto-

protective effect of PGI$_2$ during ischemia and after reperfusion is not completely understood at present. Prostacyclin could metabolically enhance the glycolytic high energy phosphate generation during myocardial ischemia. However, there are no data available on an effect of PGI$_2$ to support or refute this possibility.

In the present experiments, cardiac resting tension increased significantly during ischemia compared to control perfusion, and those hearts with high resting tensions were found to be rigid, upon tissue sampling. The resting tension of the heart is thought to reflect the free calcium level within the cardiac myocytes. In contrast, PGI$_2$ inhibited the rise in resting tension, and hearts given PGI$_2$ were not rigid after ischemia. It is possible that PGI$_2$ may have an inhibitory effect on calcium accumulation within the myocyte during ischemia. Such calcium accumulation is known to be detrimental in myocardial and liver ischemia (Chien et al., 1979; Chien et al., 1978). However, no data on the effect of PGI$_2$ on calcium metabolism are currently available.

Cardiac lysosomes includes several acid hydrolases including proteases and phospholipases. If these enzymes are released into the cytoplasm, they may contribute to the degradation of structural proteins and membrane phospholipids. During ischemia, leakage of lysosomal enzymes is reported to occur before the irreversible damage of myocardium (Wildenthal et al., 1978; Decker and Wildenthal, 1978). PGI$_2$ has been reported to be a potent stabilizer of lysosomes, possessing a much higher potency than glucocorticoids in lysosomal-enriched fractions of cat liver homogenates (Lefer et al., 1978). PGI$_2$ also stabilizes lysosomes in the isolated perfused cat liver (Araki and Lefer, 1980a) and in ischemic myocardium of intact animals (Ogletree et al., 1979). Furthermore, other agents that stabilize lysosomes were found to have cytoprotective effect in the isolated perfused cat liver (Carlson and Lefer, 1976; Araki and Lefer, 1980b). Thus, it is possible that the potent stabilizing action of PGI$_2$ on lysosomes is an important aspect of the mechanism responsible for the cytoprotective effect during ischemia observed in the present study.

None of the PGI$_2$ breakdown products studied provided a dramatic protective action against myocardial ischemia compared to the effects of prostacyclin. The major chemical breakdown product of PGI$_2$, 6-keto PGF$_{10}$, showed no significant cytoprotective or hemodynamic action during ischemia. Recently, 6-keto PGE$_1$ has been found to be a metabolite of PGI$_2$ in the liver (Wong et al., 1980), and is reported to be a potent vasodilator (Quilley et al., 1979) and inhibitor of platelet aggregation (Lee et al., 1979). Moreover, 6-keto PGE$_1$ has a beneficial effect in traumatic shock in rats (Araki and Lefer, 1979). However, the cytoprotective action of 6-keto PGE$_1$, during myocardial ischemia was minimal, exhibiting only modest preservation of cardiac function. Only perfusate CK
activity and tissue water were significantly lower than in untreated ischemic hearts. Similarly, 13,14 dihydro 6,15-diketo PGF\textsubscript{1\alpha}, a major enzymatic breakdown product of PGI\textsubscript{2}, showed no significant protection regarding CK or amino-nitrogen release during ischemia, although it partially inhibited the rise in resting tension observed during ischemia, and dF/dt recovery was significantly improved by this metabolite. Thus, a dissociation of CK release and cardiac function was observed during the infusion of 6-keto PGE\textsubscript{1} and 13,14-dihydro 6,15-diketo PGF\textsubscript{1\alpha}. At present, the mechanism of this dissociation is not known.

In summary, prostacyclin significantly counteracts the deleterious effects following reperfusion in the ischemic heart. PGI\textsubscript{2} seems to have a direct cytoprotective effect on ischemic myocardial independent of its inhibitory effect on platelet aggregation and of its coronary vasodilator action. This cytoprotection is consistent with the results of earlier studies of protection of ischemic tissue by prostacyclin (Ogletree et al., 1979; Robert et al., 1978) in the intact animal.

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Lefer AM, Ogletree ML, Smith JB, Silver MJ, Nicolau KC,
Pharmacokinetic Studies of Taurine in Bovine Purkinje Fibers

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SUMMARY Taurine (2-aminoethane sulfonic acid) is found in high concentrations in the heart, particularly in Purkinje fibers. We studied the transport of taurine in Purkinje fibers that were excised rapidly from the heart and placed in a vessel containing oxygenated Krebs-Henseleit solution (37°C). After equilibration, 4.4×10^-6 M radiolabeled taurine[14C] was added to the bath. A computer compartmental analysis of the uptake and efflux indicated the presence of two pools for uptake—a pool with a rapid kinetics $K_t$ ($t_{1/2} = 0.80$ min) and $K_2$ ($t_{1/2} = 176.30$ min). These studies suggest that Purkinje fibers have the capacity to transport taurine rapidly. Michaelis-Menten procedures showed the presence of a high affinity and a low affinity transport process. Guanidinotaurine, at a 10:1 ratio, had no appreciable effect on taurine uptake, but 3-aminoanpropane phosphonic acid decreased taurine uptake by 42.7%. Ouabain and acetylstrophanthidin ($10^{-6}$ M) inhibited taurine uptake ($K_t$) by 34% and 73%, respectively. The inhibition of the rapid component of taurine uptake suggests that $K_t$ is an energy-linked process possibly requiring Na+,K+ -ATPase. Taurine uptake in a calcium-free medium was decreased by 58%. Verapamil ($6 \times 10^{-6}$ M) decreased taurine uptake by 42%. Tetrodotoxin ($3.4 \times 10^{-6}$ M) decreased taurine uptake by 51%. The requirement of calcium and sodium for taurine uptake suggests an important relationship between taurine, calcium, and sodium in the function of fibers in the cardiac conducting system. Circ Res 47: 763-769, 1980
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