Prostacyclin: A Potentially Valuable Agent for Preserving Myocardial Tissue in Acute Myocardial Ischemia

Abstract. *Prostacyclin, a potent, naturally occurring prostaglandin exerts a variety of cardiovascular and cellular actions of potential value in acute myocardial ischemia. These properties include the reduction of systemic blood pressure without changing heart rate, the lowering of coronary vascular and total peripheral resistance, the inhibition of platelet aggregation and the concomitant formation of thromboxane B_2, and the reduction of the release of lysosomal enzymes.*

Prostacyclin (PGI_2), a novel prostaglandin that is formed by blood vessel walls, was recently discovered and identified (*1*). In its biological actions (for example, vasodilation and inhibition of platelet aggregation) PGI_2 is much more potent than prostaglandin E_1 (PGE_1). Since PGE_1 significantly prevents the extension of myocardial damage in acute myocardial ischemia (*2*), we evaluated a variety of cardiovascular and cellular properties of PGI_2 in order to determine if PGI_2 might be more useful than PGE_1 in myocardial ischemia.

For our studies, we synthesized PGI_2 by iodination and subsequent base hydrolysis of prostaglandin $F_{2\alpha}$ methyl ester using a two-step, highly efficient method (*3*). This procedure provides PGI_2 of high purity (> 95 percent) in a stable form in basic ethanolic solutions ready for biological investigations (*3*).

We anesthetized adult cats with pentobarbital sodium (30 mg/kg) given intravenously. The right carotid artery, left jugular, and right femoral veins and the trachea were cannulated. The chest was opened and positive-pressure ventilation with room air was instituted. An electromagnetic flow probe was then positioned around the root of the aorta in order to measure aortic blood flow. Electrocardiogram (ECG) standard lead 3, as well as aortic blood pressure, P_a, central venous pressure, P_r, and aortic blood flow, Q_a, were continuously recorded on a rectilinear oscillographic recorder. Total peripheral resistance (R_{tp}) was computed by the formula: $R_{tp} = (P_a - P_r)/Q_a$, where P_a and P_r are measured in millimeters of mercury, and Q_a is measured in milliliters per minute. Instantaneous heart rate was recorded from the ECG by a cardiotachometer coupler and was recorded along with the other cardiovascular variables.

Prostacyclin was infused by way of the cannulated femoral vein at rates of 0.3, 2.7, and 7.3 nmole per kilogram of body weight per minute over a 20-minute period. An equilibration of 30 minutes was used between each infusion, despite the fact that circulatory function returned to control values within 10 minutes of discontinuation of each infusion. The PGI_2 was diluted fresh in 95 percent ethanol containing 1 m*M* NaI, which was used as a vehicle control in all experiments.

Within 2 minutes of PGI_2 infusion, arterial blood pressure decreased markedly at the two higher infusion rates and only very slightly at the lowest infusion rate. As expected, heart rate increased with the decrease in arterial blood pressure. However, instead of a large increase in heart rate, as usually occurs after hypotension induced by hemorrhage (*4*), there was only an increase of about 10 beats per minute followed within 5 minutes by a decrease in heart rate of 10 to 20 beats per minute, so that heart rate equilibrated at or slightly lower than preinfusion values. This may have been due to an inhibition of sympathetic tone by PGI_2. Concomitant with these changes, mean aortic blood flow increased by 20 ± 6 percent ($N = 6$, $P < .05$) at a middle infusion rate (that is, 2.7 nmole kg^{-1} min^{-1}) and by 54 ± 25 percent ($N = 4$) at 7.3 nmole kg^{-1} min^{-1}. Figure 1 summarizes the absolute changes in mean arterial blood pressure (P_{mab}), heart rate, and total peripheral resistance for the middle infusion rate. The values were obtained 10 minutes after the onset of the PGI_2 infusion and represent steady-state values because no further changes were observed between 10 and 20 minutes. The hemodynamic profile of a decreased P_{mab}, an unchanged heart rate, and a reduction in vascular resistance are suggestive of a vasodilator effect. However, a vasodilation of this magnitude would not be expected to impair coronary blood flow. Moreover, these hemodynamic effects would be desirable in acute myocardial ischemia because they resulted in a decrease of 15 ± 4 percent in cardiac work ($P < .02$). The lowest infusion rate did not exert significant effects on any of the cardiovascular variables studied, whereas the highest infusion rate decreased P_{mab} by 68 ± 6 mm-Hg ($P < .02$). Infusion of the PGI_2 vehicle did not result in any significant changes in the variables measured.

In addition to the studies of intact animals just described, we studied the effects of PGI_2 in isolated tissues under controlled conditions. We chose the following cat tissue preparations as our test systems in vitro: (i) isolated perfused coronary arteries for coronary vasoactivity, (ii) isolated papillary muscles for cardiac muscle contractility, (iii) large granule fractions (LGF) of liver as a lysosomal-rich preparation for lysosomal membrane stability, and (iv) platelet-rich plasma for platelet aggregation.

Coronary arteries were isolated from cat hearts as described (*5*) and perfused under constant flow (that is, 35 ml/min) with oxygenated Krebs-Henseleit solution (*p*H 7.3) specifically modified for cat tissues (*6*). Perfusion pressure, as an index of vasoactivity, was continuously recorded by means of Statham pressure transducers and an oscillographic recorder. After an initial 30- to 60-minute equilibration period, the arteries were tested with 25 m*M* KCl, a constrictor of these vessels, as well as with adenosine (150 n*M*), a dilator of coronary arteries. Concentration-response relationships of PGI_2 at concentrations from 1 to 500 n*M* are summarized in Fig. 2. Concentrations of 1 to 10 n*M* did not significantly alter coronary perfusion pressure. However, 50 to 500 n*M* PGI_2 induced a progressive concentration-dependent decrease in coronary perfusion pressure indicative of coronary vasodilation ($P < .02$ to $< .001$). This dilation is

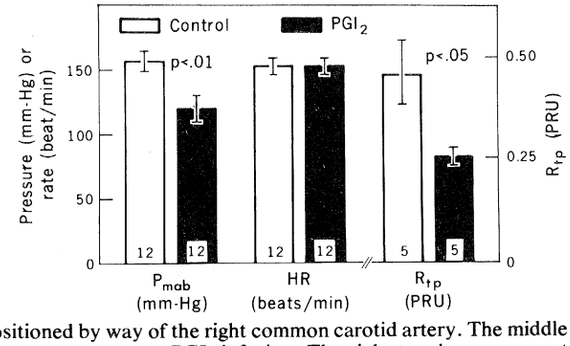

Fig. 1. Hemodynamic response in cats 10 minutes after intravenous infusion of PGI_2 (2.7 nmole kg^{-1} min^{-1}). Open bars indicate preinfusion values; solid bars indicate values attained after 10 minutes of PGI_2 infusion (means ± standard error). Numbers within bars indicate the number of cats in which responses were measured. The left two bars represent values of mean arterial blood pressure (P_{mab}) measured by intraaortic catheters positioned by way of the right common carotid artery. The middle two bars indicate the heart rate (*HR*) response to PGI_2 infusion. The right two bars represent values for total peripheral resistance (R_{tp}) measured in peripheral resistance units.

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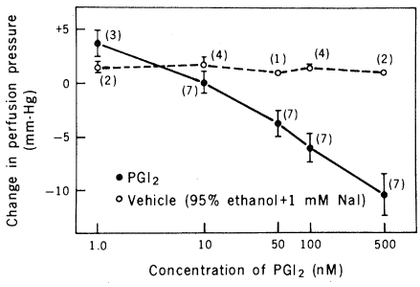

Fig. 2. Responses of isolated perfused cat coronary arteries to PGI_2. Cat coronary arteries (1.5 to 2.0 cm long) were perfused at constant flow (35 ml/min) with recirculating oxygenated (95 percent O_2 plus 5 percent CO_2) Krebs-Henseleit solution. Coronary perfusion pressure was continuously monitored as PGI_2 or an appropriate dilution of the PGI_2 solvent (95 percent ethanol plus 1 m*M* NaI) was added to the perfusate. Peak pressure responses were observed within 5 minutes of drug addition. Solid circles represent mean changes in perfusion pressure (ordinate) following addition of PGI_2 in final bath concentrations shown on the abscissa. Open circles show pressure responses to appropriate dilutions of the PGI_2 vehicle. Numbers in parentheses indicate number of vessels tested at each point.

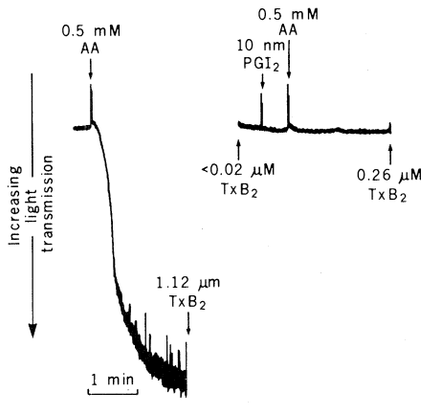

Fig. 3. Typical platelet aggregation tracings showing inhibition of platelet aggregation and of thromboxane B_2 (*TxB*$_2$) formation by 10 n*M* prostacyclin (*PGI*$_2$) after the addition of arachidonic acid (*AA*) to platelet-rich plasma (*PRP*). Cat PRP was prepared by centrifugation of citrated (1:10 volume, 3.8 percent trisodium citrate) whole cat blood at 200*g* for 15 minutes. The PRP was first incubated at 37°C. Arachidonic acid (0.5 m*M*) was added to 0.5 ml of stirred PRP. After 2 minutes, a 50-μl portion of PRP was assayed for thromboxane B_2 (*14*).

greater than that produced by an equimolar concentration of PGE_1 (*7*), and is not significantly different from the response to an equimolar concentration of adenosine, one of the most potent coronary vasodilators known (*8*).

At concentrations from 1 to 1000 n*M*, PGI_2 did not exert significant inotropic effects in the electrically driven isolated cat papillary muscles (*9*). The muscles responded with increases of 100 percent to isoproterenol and decreases of 60 percent to myocardial depressant factor (*9*) given at the end of the experiment, demonstrating that they were responsive to both positive and negative inotropic stimuli. However, 50 n*M* PGI_2 did not alter contractile force. Responses to 10 to 100 n*M* PGI_2 averaged a 0.4 ± 0.7 percent increase (not significant) for six muscles. Thus, PGI_2 does not possess significant direct cardiostimulatory activity in isolated cat cardiac tissue. This finding suggests that the increase in cardiac output in response to PGI_2 observed in the intact cat is primarily due to systemic vasodilation and to indirect cardiac effects (that is, increased cardiac ejection in the face of an increased venous return).

The LGF of cat liver homogenates were prepared as described (*10*), 1 g of liver tissue being used for each 6 ml of 0.25*M* sucrose. The homogenate was centrifuged at low speed to remove cell debris, then centrifuged at 15,000*g* for 30 minutes at 4°C. The pellet from this centrifugation, washed twice with 0.25*M* sucrose, was designated the LGF. Suspensions of LGF were incubated at 37°C in Krebs-Henseleit solution either in the presence of PGI_2 (50 n*M*) or ethanol-NaI vehicle for 30 minutes. At the end of this incubation period, the flasks were placed on ice, and the 30-minute samples were collected for assay of the lysosomal enzymes β-glucuronidase and cathepsin D according to established spectrophotometric procedures (*11*). The percentage of release of each lysosomal hydrolase over the 30-minute incubation period was calculated by comparison of enzyme assays in the presence and absence of 0.1 percent Triton X-100 (that is, a nonionic detergent that rapidly releases all lysosomal hydrolase activity). With PGI_2 at 50 n*M*, a concentration computed to be approximately equivalent to the middle infusion rate in the intact cats, there was a significant decrease in both β-glucuronidase and cathepsin D release. In this regard, suspensions of LGF released 33.9 ± 2.2 percent of their total β-glucuronidase in the absence of PGI_2 (that is, in the presence of the ethanol-NaI vehicle), whereas they released only 26.1 ± 2.3 percent of their total in the presence of 50 n*M* PGI_2, a difference of 7.8 ± 1.3 percent ($P < .02$). Similarly, PGI_2 reduced the rate of cathepsin D release in suspensions of LGF from 24.1 ± 1.5 percent to 12.4 ± 2.9 percent ($P < .01$), a difference of 11.7 ± 2.4 percent. These findings provide strong support for a lysosomal stabilizing effect of PGI_2. Moreover, PGI_2 is five to ten times more potent than PGE_1 in preventing lysosomal hydrolase release (*11*) and thus appears to be a potent membrane-stabilizing agent, at least in suspensions of liver lysosomes.

We also tested the ability of PGI_2 to inhibit arachidonic acid–induced platelet aggregation in cat platelet-rich plasma using the method of Born (*13*) to assess platelet aggregation. Figure 3 shows that PGI_2 (10 n*M*) abolished platelet aggregation in response to 0.5 m*M* arachidonic acid as evidenced by the amplitude of the platelet aggregation curves. At the same time, PGI_2 suppressed the production of thromboxane A_2 by platelets as measured by specific radioimmunoassay of its stable metabolite, thromboxane B_2, possibly by an adenosine 3′,5′-monophosphate–mediated inhibition of cyclooxygenase (*14*). Also, PGI_2 was a potent inhibitor of the aggregation of cat platelets induced by prostaglandin G_2, adenosine diphosphate, or collagen.

Prostacyclin (at 2.7 nmole kg^{-1} min^{-1}) decreased systemic blood pressure, total peripheral resistance, and cardiac work in the anesthetized cat without changing heart rate. This prostaglandin also decreased coronary vascular resistance (that is, by decreasing coronary perfusion pressure at a constant coronary flow) suggesting that it improves coronary flow. If these effects occurred to a significant degree in myocardial ischemia, they would markedly improve myocardial oxygen supply relative to myocardial oxygen demand. The net result of these effects would be to retard the spread of ischemic damage and thus reduce the size of the developing myocardial infarct (*15*).

In addition to these hemodynamic actions, PGI_2 stabilizes lysosomal membranes, antagonizes platelet aggregation, and inhibits platelet thromboxane production. These cellular effects may be of great significance in preventing the spread of ischemic damage within the myocardium. In this regard, myocardial lysosomal disruption occurs within the first 2 to 5 hours of acute myocardial ischemia (*16*), and lysosomal hydrolases once released into the circulation are known to impair the reserve capacity of the heart as well as curtail coronary flow (*17*). Inhibition of platelet aggregation is a very important effect of PGI_2 since platelet aggregation is a vital factor in thrombotic processes, and vascular thrombosis may be an important response to ischemia in the coronary vasculature. Finally, inhibition of thromboxane generation by PGI_2 is another important feature of the anti-ischemic profile of this prostaglandin since thromboxane A_2 is thought to induce constriction of the coronary vasculature

(*18*). This constriction may provoke coronary vasospasm which could trigger an acute ischemic crisis. Thus, PGI_2 may be useful in the prevention of secondary ischemic episodes once an initial ischemic event occurs in the heart.

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Size and Shape of the Lateral Intercellular Spaces in a Living Epithelium

Abstract. *The lateral intercellular spaces of* Necturus *gallbladder epithelium were seen and measured while the living tissue was perfused in a new chamber. The compliance of the lateral cell membranes was calculated from the measured pressure-volume characteristics of the lateral intercellular spaces.*

The lateral intercellular spaces of fluid-transporting epithelia have been assigned a major role in the transepithelial movement of water. On the basis of electron micrographs of rabbit gallbladder, it was concluded that the size of the lateral spaces was directly related to the rate of isotonic fluid transport by the gallbladder and that the interspaces represent the final common path for transported fluid (*1*). Several aspects of these results have been called into question. It has been suggested that the dimensions of the lateral spaces are such that current mathematical models fail to predict an isotonic absorbate (*2*). Fredericksen and Rostgaard (*3*) even questioned the existence of dilated lateral intercellular spaces in living preparations and suggested that the fixation and embedding procedures preparative to electron microscopy produced artificially dilated spaces because of cell shrinkage. Other authors (*4*) reported that fixation procedures may alter intercellular space geometry, making the accuracy of morphometric data questionable.

The conclusions of many investigators about the role of the lateral spaces are heavily dependent on the accuracy of measurements of the spaces in electron micrographs. Since there is controversy about the relevance of intercellular dimensions in fixed preparations to the living tissue, we developed a method for light microscopic observation of the spaces in a living, flat epithelium. This system makes it possible to continuously measure the width or cross-sectional area of the lateral intercellular spaces at any distance between the tight junction and the basement membrane. Simultaneously, we can measure the epithelial electrical resistance, pass current across the epithelium, vary and measure the hydrostatic pressure across the epithelium, and rapidly alter the composition of the fluids bathing either surface of the epithelium. Thus it is possible to perform most of the experiments currently utilized for studying epithelia while monitoring the size and shape of the intercellular spaces. *Necturus* gallbladder was chosen for the initial experiments because of its relatively large cell size (15 to 25 μm in diameter) (*5*).

The chamber for the gallbladder, shown in an exploded view in Fig. 1, is a miniature Ussing chamber (*6*) whose dimensions are chosen to satisfy the optical requirements of the microscope lens system. The overall chamber thickness is 1.1 mm and the tissue is within 75 μm of the inner surface of the bottom cover glass. The chamber was modified from a Dvorak-Stotler tissue culture chamber (*7*). The tissue is held mucosal side downward between concentric rings that are placed inside a plastic spacer. The bottom of the spacer is composed of two laminated sheets of mica and the bottom cover glass (Fig. 1). The channel in the lower mica forms the mucosal bath (*8*). The serosal bath is formed by fluid above the smallest tissue-holding ring. Before assembly, the topmost mica surface was painted lightly with liquid Sylgard 184 (Dow Chemical Co., Midland, Michigan) to achieve an electrical seal of the tissue to the mica around the center hole. The size of the center hole in the uppermost mica sheet determines the area of tissue exposed to the mucosal bath. We tried several sizes, ranging in diameter from 75 μm to 1.5 mm. The optimum size was a 1.5-mm-diameter hole, determined by the electrical properties of the tissue and mechanical considerations. Thus the exposed tissue has an area of 1.8×10^{-2} cm^2.

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