**BYPASS CONDUIT VESSEL
WALL BIOLOGY
SUBSTANTIALLY
INFLUENCES DOWNSTREAM
MYOCARDIAL CONTRACTILE
RESPONSE TO INJURY
FROM ISCHEMIA AND
REPERFUSION**

Coronary vascular intraluminal release of endogenous endothelium-derived substances, such as prostacyclin, may affect downstream cardiac myocyte contractile function. With a "chronic" canine model of endothelialized and deendothelialized internal thoracic artery coronary grafts, we tested the hypothesis that higher basal release of endothelium-derived prostacyclin in internal thoracic artery bypass conduit effluent accelerates functional recovery of postischemic stunned myocardium in the intact circulation. Eleven dogs underwent left internal thoracic artery-left circumflex artery bypass, and the proximal circumflex artery was then ligated. Internal thoracic artery conduit endothelium was denuded by balloon catheter in five dogs before grafting and left intact in six dogs. After 7 days, awake dogs were studied to measure myocardial segment length in the circumflex region with ultrasonic dimension transducers, left ventricular pressure with micromanometers, and circumflex artery flow with an ultrasonic flow probe. Regional contractile function was quantified by the area beneath the linear preload recruitable stroke work relationship at baseline and at intervals after a 15-minute circumflex graft occlusion followed by 3 hours of reperfusion. Heart rate, left ventricular peak pressure, left ventricular end-diastolic pressure, left ventricular peak first derivative of pressure (dP/dt), and circumflex flow were similar (all p not significant) in endothelialized and nonendothelialized dogs during ischemia and reperfusion. Ischemia reduced the preload recruitable stroke work relationship to $44\% \pm 35\%$ of control values ($p < 0.01$) in endothelialized dogs and to $47\% \pm 18\%$ of control values in nonendothelialized dogs ($p < 0.01$) at 15 minutes of reperfusion, indicating a similar (p not significant) initial degree of injury. During 3 hours of reperfusion, the preload recruitable stroke work relationship returned to $51\% \pm 17\%$ of control values in endothelialized dogs but to only $35\% \pm 20\%$ of control values in nonendothelialized dogs ($p < 0.02$). Basal intraluminal release of endogenous prostanoids in excised internal thoracic artery conduits was subsequently quantified by ex vivo bioassay of vasoactive properties of conduit effluent on normal coronary artery smooth muscle. Endothelialized conduits induced greater smooth muscle relaxation than did nonendothelialized conduits (67% vs 23%), and this increased relaxation by endothelialized conduits was eliminated by indomethacin, a blocker of prostanoid synthesis. These data indicate that coronary bypass conduit endothelium-derived substances, such as prostacyclin, significantly influence downstream myocardial contractile response to ischemia and reperfusion, independent of alterations in coronary flow in the intact circulation. (J THORAC CARDIOVASC SURG 1996;111:62-73)

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Superior performance of the internal thoracic artery (ITA) for coronary artery bypass grafting is the result of intrinsic biologic properties of the ITA conduit. In addition to anatomic and geometric advantages, which result in a more favorable flow velocity profile, lower shear stresses,¹⁻³ and greater resistance to smooth muscle intimal hyperplasia and atherosclerotic changes,^{4,5} there are also important differences in production of the endothelium-derived polypeptide prostacyclin by ITA and saphenous vein grafts. Endogenous endothelial release of prostacyclin, a coronary vasodilator and an inhibitor of platelet aggregation and platelet-induced vasoconstriction, is significantly greater in human ITA than in saphenous vein.⁶⁻⁹ These and perhaps other characteristics of conduit vessel wall biology probably also contribute to improved performance of the ITA conduit in the coronary circulation.

Previously, substantial attention has been directed toward characterization of how intraluminal release of endothelium-derived prostacyclin may affect the local coronary vascular environment to promote vasodilation and retard atherosclerotic degeneration.¹⁰ Increasing evidence now indicates that coronary vascular intraluminal release of endothelium-derived substances may also exert significant endocrine effects on downstream cardiac myocyte contractile function and may act to influence myocellular tolerance to ischemic and reperfusion injury.¹¹⁻¹⁷ Stimulation of endothelium-derived nitric oxide has been demonstrated to have detrimental effects on contractile performance of dysfunctional myocytes.¹³⁻¹⁷ Conversely, stimulation of endogenous prostacyclin synthesis^{11,12,18} and administration of exogenous prostacyclin¹⁹⁻²¹ or prostacyclin analog²²⁻²⁴ have been shown to have beneficial cardioprotective effects against myocardial ischemia and reperfusion injury and to attenuate postischemic myocardial stunning. In addition to its actions as a vasodilator and platelet suppressant, prostacyclin is known to oppose the effects of leukocyte adhesion molecules,²⁵ to inhibit the transmigration of neutrophils into reperfused myocardium, and to inhibit the neutrophil oxidative burst.^{21,23,24} The attenuation of neutrophil-mediated free-radical injury during reperfusion is thought to be a potential mechanism by which prostacyclin may enhance postischemic myocardial functional recovery. Endogenous ITA conduit endothelium-derived factors such as prostacyclin could be released in sufficient concentrations in vivo to exert significant biologic effects and influence downstream myocellular con-

tractile function in the intact circulation, but this has not previously been well defined.

We therefore undertook a study that used endothelialized and deendothelialized ITA conduits grafted to a proximally ligated coronary artery in the "chronic" canine model to develop a suitable experimental model for the investigation of bypass conduit endothelium-mediated myocardial events in the intact circulation. We tested the hypothesis that higher basal release of endothelium-derived prostacyclin in ITA bypass conduit effluent may have important effects on downstream myocellular contractile function and act to accelerate functional recovery of postischemic stunned myocardium.

Methods

Experimental protocol

ITA grafting and instrumentation. Eleven adult mongrel dogs (27 ± 3 kg) were anesthetized with pentobarbital (30 mg/kg administered intravenously), intubated, and mechanically ventilated with oxygen. Under sterile conditions, a left thoracotomy was performed through the fourth intercostal space and the left ITA pedicle was dissected from the chest wall from its origin at the left subclavian artery to the fifth intercostal space. Heparin (100 U/kg) was administered intravenously and the distal ITA was ligated and divided so that the in situ ITA graft length was approximately 12 cm. The pericardium was opened and the proximal portion of the left circumflex coronary artery (LCX), beginning at its origin at the left main coronary artery, was exposed for a length of approximately 1.5 cm in the atrioventricular groove beneath the left atrial appendage. Dogs then underwent coronary artery bypass grafting with the distal end of the in situ ITA graft anastomosed to the proximal LCX. To avoid extracorporeal bypass, a temporary intraluminal coronary shunt was used to maintain LCX myocardial blood flow during anastomosis of the ITA to the coronary artery in the beating heart, as described in detail elsewhere.²⁶ In brief, the proximal end of a silicone elastomer catheter (0.062-inch i.d. Silastic catheter; Dow Corning Corp., Medical Materials, Midland, Mich.) was inserted through a small arteriotomy into the right ITA, and pulsatile blood flow through the shunt was visually confirmed. Lidocaine (1 mg/kg) was administered intravenously, and the proximal LCX was ligated immediately beyond its origin from the left main coronary artery. An oblique arteriotomy was made in the LCX wall, with a distal suture loop around the LCX placed under tension to avoid back bleeding. The distal end of the silicone elastomer shunt was passed through the LCX arteriotomy to just beyond the looped suture but proximal to the origin of the first obtuse marginal branch of the LCX. Ties were secured and a third silk suture was then positioned proximal to the tip of the shunt to prevent back bleeding when the LCX was opened. The shunt was unclamped and blood flow was reestablished into the distal LCX. Reestablishment of blood flow to the LCX region was visually confirmed by immediate resolution of myocardial cyanosis and resump-

tion of regional myocardial systolic contraction. On average, coronary artery occlusion lasted less than 60 seconds. A longitudinal arteriotomy was then made in the LCX over the shunt, and the distal end of the IMA was anastomosed to the LCX in a bloodless field with running 7-0 polypropylene sutures. After completion of the anastomosis, the shunt was removed and the LCX was ligated just proximal to the anastomosis. Blood flow into the distal LCX was then completely dependent on the ITA graft.

In six of 11 dogs, the ITA was not probed or instrumented in any way before anastomosis and the ITA endothelium was left intact and undisturbed. These dogs were designated the endothelialized (ENDO) group. In the other five of 11 dogs, designated the nonendothelialized (NoENDO) group, before anastomosis of the ITA graft the ITA endothelium was denuded by insertion of a balloon catheter (5F) into the ITA followed by gentle balloon inflation and withdrawal of the catheter over the entire length of the ITA graft. ITA endothelial denudation by this method, described elsewhere in detail,²⁷⁻²⁹ was subsequently confirmed in representative dogs by scanning electron microscopic examination of the luminal surface of excised ITA grafts at the completion of the study. The presence of an intact endothelium was also confirmed in representative dogs in the ENDO group by scanning electron microscopy. Dogs were allocated to ENDO and NoENDO groups in a random manner.

In all dogs, pneumatic cuff occluders were placed around the superior and inferior venae cavae and around the distal ITA graft just proximal to the LCX anastomosis. An ultrasonic flow probe (inner diameter 2 mm; Transonic Systems, Inc., Ithaca, N.Y.) was placed around the LCX just distal to the anastomosis to measure LCX coronary blood flow. A bipolar pacing electrode was sutured to the surface of the left atrium. A silicone rubber tube (inner diameter 2.6 mm, outer diameter 4.9 mm; Dow Corning) was implanted in the base of the left atrial appendage for later passage of a micromanometer into the left ventricle. A similar tube with multiple side holes was positioned in the pleural space adjacent to the left ventricle for measurement of pleural pressure. Pairs of miniature subepicardial pulse-transit ultrasonic dimension transducers (outer diameter 1.5 mm; Physiological Monitoring Systems Group, Durham, N.C.) were implanted into the left ventricle to measure myocardial segment dimension. One pair was positioned in the distribution of the LCX distal to the ITA graft anastomosis and a second pair was positioned in the distribution of the left anterior descending coronary artery. Each pair was aligned 10 to 15 mm apart along the minor axis circumference of the left ventricle midway between apex and base. Cables and tubes were passed through the chest wall into a dorsal subcutaneous pouch, the pericardium was left opened, and the thoracotomy was closed in layers. Each dog was allowed to recover for 7 days, receiving intramuscular injections of butorphanol tartrate (0.5 mg) for pain and penicillin G (Bicillin) (1.25 MU) and oral doses of aspirin (81 mg daily).

Data acquisition. After 7 days, dogs were studied in the conscious state lying quietly on their right side. Intramuscular morphine (0.7 mg/kg) was given and cable connec-

tors were exteriorized through a small skin incision with 1% lidocaine local anesthesia. Ultrasonic dimension transducers were connected to a sonomicrometer (Davis Associates, Durham, N.C.). Micromanometers were passed through the implanted silicone rubber tubes into the left ventricle and into the pleural space adjacent to the left ventricle. The left atrium was paced at a constant rate. Lidocaine (1 mg/kg) was administered, and 10 minutes later pressure, dimension, and coronary flow data were recorded. The ITA graft to the LCX was then occluded for 15 minutes and reperfused. Lack of flow during LCX graft occlusion and restoration of flow after occluder release were confirmed by flow probe measurement. At the end of the 15-minute LCX occlusion, the occluder was gradually released, initially allowing LCX flow to return only to control preischemic values to attenuate the reperfusion hyperemic response and to control for any potential differences between ENDO and NoENDO groups in LCX reperfusion flow.

Data were acquired at control before ischemia, just before coronary reperfusion, and at 15, 30, 60, 90, 120, 150, and 180 minutes after reperfusion. At each time point, static data were recorded at a constant heart rate and data were also recorded over a range of left ventricular end-diastolic volumes produced by transient (5- to 10-second) occlusion of the venae cavae for 10 to 25 cardiac cycles. At the conclusion of 3 hours of reperfusion, dogs were killed by deep barbiturate anesthesia and the hearts and ITA grafts were excised.

In five dogs (three ENDO, two NoENDO) basal intraluminal release of endogenous prostanoid synthesis by ITA conduits was quantified by ex vivo bioassay of the vasoactive properties of ITA conduit effluent, as described elsewhere in detail.³⁰ In brief, excised ITA grafts were immediately immersed in cool, oxygenated physiologic salt solution of the following composition (in millimoles per liter): sodium chloride, 118.3; potassium chloride, 4.7; magnesium sulfate, 1.2; potassium phosphate, 1.22; CaCl₂, 2.5; sodium hydrogen carbonate, 25.0; and glucose, 11.1. The ITA was gently cleaned of surrounding tissue and care was taken not to touch the luminal surface. ITA conduits were mounted on a stainless steel cannula and perfused with oxygenated physiologic solution at 37° C at a constant flow rate (5 ml/min). Beneath the ITA conduits, isolated 3 mm rings of coronary artery smooth muscle from which the endothelium had been mechanically removed (obtained from a control dog not otherwise operated on) were suspended in a 25 ml organ chamber filled with oxygenated physiologic solution at 37° C. Rings were suspended by two stainless steel clips anchored and connected to a strain gauge (Statham Gould UC2; Spectramed Inc., Critical Care Division, Oxnard, Calif.) for measurement of isometric force. The coronary vessel rings were first superfused for 60 minutes with control solution and stretched in stepwise fashion to optimal tension (10 g). Relaxation of bioassay rings in response to direct perfusion with ITA conduit effluent was examined during bioassay ring contraction caused by prostaglandin F_{2α}. The absence of endothelium on bioassay rings was confirmed by the lack of relaxation to calcium ionophore A23187 (10⁻⁶ mol/L). Relaxation of bioassay rings was also measured after stimulated release of ITA conduit

vasoactive substances elicited by 200 μ l of calcium ionophore A23187 (10^{-6} mol/L) injected into the perfusate above the ITA conduit, both with and without addition of indomethacin (10^{-5} mol/L), a blocker of endogenous prostanoid synthesis, to the ITA perfusate. The degree of ring relaxation is indicative of the quantity of intraluminal release of endogenous vasoactive substance produced by the ITA conduit. Relaxation is expressed as a percentage of the maximal contraction achieved by prostaglandin $F_{2\alpha}$. ITA grafts from dogs in the ENDO and NoENDO groups that were not bioassayed for endogenous prostanoid synthesis were processed for scanning electronmicrographic examination of the ITA luminal surface.

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication no. 85-23, revised 1985). The procedures and handling of animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Mayo Foundation.

Data analysis. Pressure, dimension, and flow data were filtered with a 50 Hz low-pass analog filter and digitized at an 8-channel sweep speed of 200 Hz by an analog-to-digital converter (model 5025MF; ADAC, Woburn, Mass.). The analog-to-digital conversion time per channel was 30 msec, creating a phase delay between channels of less than 4.5 degrees. After data were collected and stored on a hard disk by personnel computer (Reason Technology Inc., Minneapolis, Minn.), data analysis was accomplished on a microcomputer (DEC Vaxstation 3100; Digital Electronic Corporation, Maynard, Mass.) by means of commercial interactive software (Davis Associates) and software developed in our laboratory. Instantaneous left ventricular transmural pressure was calculated as the difference between left ventricular pressure and pleural pressure. The first derivative of left ventricular transmural pressure (dP/dt) was determined from a running 5-point polyorthogonal transformation of the digitized ventricular pressure waveform. Cardiac cycles were automatically defined with dP/dt criteria. Regional left ventricular systolic contractile function was quantified by the linear and load-independent preload recruitable stroke work (SW) relationship, an analysis of ventricular myocardial segment ability to generate as a function of end-diastolic length (EDL). This analysis is a highly sensitive, useful parameter to assess regional myocardial dysfunction after acute ischemic injury, as described elsewhere in detail.^{31, 32} In brief, regional left ventricular SW values were calculated from instantaneous transmural pressure (P) and dimension (L) data for each cardiac cycle as follows:

$$SW = \int P \cdot dL \quad (1)$$

Data from each vena caval occlusion were fitted to the following equation:

$$SW = M_w (EDL - L_w) \quad (2)$$

relating SW to EDL where M_w is the slope and L_w is the x-axis intercept. Preload recruitable work area (PRWA) was defined as the area under the SW versus EDL regression line and was calculated as follows:

$$PRWA = M_w/2 \times (L_{w \max} - L_w)^2 \quad (3)$$

where $L_{w \max}$ was the maximal x-intercept value obtained for a given myocardial segment over the entire experiment. Changes in ventricular contractile function were reflected as changes in M_w and L_w and by changes in PRWA, which reflected changes in both slope, M_w , and x-intercept, L_w (Fig. 1). For each dog, M_w , L_w , and PRWA were determined from equations 2 and 3 for both the LCX and left anterior descending coronary artery regions for each vena caval occlusion. Mean values were calculated for three to five vena caval occlusions performed at baseline before ischemia produced by LCX occlusion and after ischemia at 15, 30, 60, 90, 120, 150, and 180 minutes of reperfusion. Standard hemodynamic data (heart rate, left ventricular peak pressure, left ventricular end-diastolic pressure, left ventricular peak dP/dt, and LCX flow) were also acquired.

All data are presented as mean $\pm$ standard deviation except as otherwise noted. Statistical analyses were performed with paired and unpaired t -tests and one- and two-way analyses of variance with the Newman-Keuls test to localize differences as appropriate. Statistical significance was accepted at $p < 0.05$.

Results

Scanning electron microscopy of ITA grafts.

Scanning electron microscopic views of the luminal surface of an ITA graft from representative dogs in the ENDO and NoENDO groups are shown in Fig. 2. In grafts from the ENDO group (Fig. 2, A) the ITA endothelium is intact and endothelial cell borders are well demarcated, with elevated central portions of the cells indicative of the presence of nuclei, and there is minimal widening of intracellular borders. In the grafts from the NoENDO group (Fig. 2, B) the ITA intimal surface is denuded of endothelial cells, with exposure of the subendothelial matrix, confirming ITA deendothelialization accomplished by balloon catheter.

Circumflex coronary artery blood flow. At baseline before LCX graft occlusion, mean LCX coronary blood flow was 60 ± 4 ml/min in the ENDO group and 61 ± 21 ml/min in the NoENDO group (p not significant). After release of the LCX graft occlusion, early reperfusion flows were similar (p not significant) in the ENDO and NoENDO groups and did not differ (p not significant) from control preischemic values in either group. LCX coronary blood flows did not differ (all p not significant) at any time during reperfusion in the ENDO and NoENDO groups (Fig. 3).

Fig. 1. **A**, Characteristic plot of instantaneous left ventricular transmural pressure (*LVP*) and myocardial segment dimension in the region of the circumflex artery for cardiac cycles over a range of end-diastolic volumes produced by transient vena caval occlusion. **B**, Expressing left ventricular regional SW as a function of EDL for each cardiac cycle (equations 1 and 2 in text) yields a highly linear relationship precisely quantifiable by a slope, M_w , and an *x*-axis intercept, L_{w1} . **C**, Computation of PRWA as area under segmental SW versus EDL relationship (equation 3 in text) for two different SW versus EDL relationships in the same dog at different times. L_{w1} and L_{w2} are relationship *x*-axis intercepts for individual study times and L_{wmax} is the maximal value of L_w for the entire experiment.

Hemodynamics during regional ischemia and reperfusion. As summarized in Fig. 4, the effects of LCX graft occlusion and reperfusion on heart rate, left ventricular peak pressure, left ventricular end-diastolic pressure, and left ventricular peak dp/dt were similar (all *p* not significant) in the ENDO and NoENDO groups, indicating no differences in global hemodynamics between the two groups.

Regional myocardial contractile function. At baseline before LCX graft occlusion, myocardial contractile function in the LCX region did not differ in the ENDO and NoENDO groups. The slope, M_w , and *x*-intercept, L_{w1} , of the LCX regional preload recruitable SW relationship, and PRWA were similar (all *p* not significant) in ENDO and NoENDO groups (Table I).

In both the ENDO and NoENDO groups 15 minutes of LCX graft occlusion resulted in significant depression of myocardial contractile function in the LCX region, reflected as a significant reduction in regional PRWA (Table I). LCX graft occlusion reduced LCX regional PRWA to $44\% \pm 35\%$

of the preischemic control value in the ENDO group and to $47\% \pm 18\%$ of the preischemic control value in the NoENDO group (both *p* < 0.01) at 15 minutes of reperfusion. The decrease in PRWA measured in the LCX region after ischemia at 15 minutes of reperfusion was similar (*p* not significant) in the ENDO and NoENDO groups, indicating a similar initial degree of ischemic injury measured during early reperfusion in the two groups of dogs (Table I).

During subsequent reperfusion, however, there was an accelerated recovery of LCX regional contractile function in the ENDO group compared with that seen in the NoENDO group (*p* < 0.02; (Fig. 5). By 3 hours of reperfusion, LCX regional PRWA returned to $51\% \pm 17\%$ of preischemia control values in the ENDO group but remained depressed at only $35\% \pm 20\%$ of preischemia control values in the NoENDO group.

Myocardial contractile function in the distribution of the left anterior descending coronary artery, as assessed by M_w , L_{w1} , and PRWA of the regional

Fig. 2. **A**, From a representative dog in the ENDO group, the intraluminal surface of a noninstrumented ITA graft 7 days after anastomosis to the circumflex coronary artery. The endothelium is intact, with well demarcated endothelial cell borders, elevated central portions of the cells indicative of the presence of nuclei, and minimal widening of intracellular spaces (Scanning electron micrograph; original magnification $\times 1600$). **B**, From a representative dog in the NoENDO group, the intraluminal surface of an ITA graft 7 days after balloon catheter deendothelialization and anastomosis to the circumflex coronary artery. The intimal surface is denuded of endothelial cells and the subendothelial matrix is exposed (scanning electron micrograph; original magnification $\times 1600$).

preload recruitable SW relationship, did not differ (all p not significant) in the ENDO and NoENDO groups at baseline before LCX graft occlusion. LCX graft occlusion resulted in no significant depression

of contractile function in the region of the left anterior descending coronary artery; M_w , L_w , and PRWA remained similar (all p not significant) to control preischemic values in both the ENDO and

Fig. 3. Effects of circumflex graft occlusion and reperfusion on circumflex coronary flow in ENDO and NoENDO groups. Values are mean $\pm$ standard error of the mean. All values p not significant (ns) versus control preischemia value by one-way analysis of variance for repeated measures for both ENDO and NoENDO groups. Difference between ENDO and NoENDO groups tested by two-way analysis of variance for repeated measures.

NoENDO groups. There were also no differences (p not significant) between the ENDO and NoENDO groups in contractile function in the control left anterior descending region at any time during LCX reperfusion.

Bioassay of ITA graft effluent. Ex vivo bioassay of excised ITA conduit effluent demonstrated that effluent from dogs in the ENDO group induced substantially greater relaxation of coronary artery smooth muscle rings than did conduit effluent from dogs in the NoENDO group (Fig. 6). The increased smooth muscle relaxation induced by conduit effluent from dogs in the ENDO group was substantially reduced by addition to conduit perfusate of indomethacin, a blocker of endogenous prostanoid synthesis. This indicates greater intraluminal release of prostacyclin or of a vasoactive prostanoid similar to prostacyclin in the endothelialized conduit.

Discussion

These data in the conscious canine model of regional myocardial ischemia and reperfusion demonstrate that bypass conduit vessel wall biology significantly influences downstream myocardial contractile response to ischemia and reperfusion. Myocardial regions perfused by endothelium-containing ITA conduits demonstrate accelerated recovery of

contractile function after being subjected to a 15-minute period of coronary artery occlusion and 3 hours of reperfusion. This difference in myocardial functional recovery was not related to differences in total regional coronary blood flow during reperfusion or to differences in global hemodynamics during ischemia and reperfusion. The enhanced functional recovery of myocardial regions perfused by endothelium-containing conduits was associated with elevated levels of prostacyclin or a prostacyclin-like substance in bypass conduit effluent.

In this study, we used balloon catheter endothelial denudation of ITA conduits in a chronic canine model of coronary bypass to the circumflex artery with proximal ligation of the native coronary vessel to characterize downstream effects of endothelium-derived factors, in particular prostacyclin, on myocardial function in the intact circulation. Our intent was to analyze the contractile response to ischemia and subsequent reperfusion in myocardial regions perfused by conduits with disparately high and low levels of basal endogenous intraluminal release of endothelium-derived prostacyclin. Previous work has demonstrated the high capacity for endogenous prostacyclin synthesis by canine^{28,33} and human ITA endothelium.^{6,7} Probing of the canine ITA to disrupt endothelial integrity has been shown to result

Fig. 4. Effects of circumflex graft occlusion and reperfusion on heart rate, left ventricular (LV) peak pressure, left ventricular end-diastolic pressure, and left ventricular peak dP/dt in ENDO and NoENDO groups. Values are mean $\pm$ standard error of the mean. All values p not significant (ns) versus control preischemia value by one-way analysis of variance for repeated measures for both ENDO and NoENDO groups. Differences between ENDO and NoENDO groups tested by two-way analysis of variance for repeated measures.

Table 1. Effects of circumflex graft occlusion and reperfusion on myocardial contractile function in the circumflex region quantified by slope, M_w , x-intercept, L_w , and PRWA in ENDO and NoENDO groups

	Preischemia	Reperfusion							p^*
		15 min	30 min	60 min	90 min	120 min	150 min	180 min	
M_w (erg $\cdot$ cm $^{-3}$ $\cdot$ 10 3)									
ENDO	125 $\pm$ 34	126 $\pm$ 55	142 $\pm$ 38	140 $\pm$ 36	133 $\pm$ 48	128 $\pm$ 35	113 $\pm$ 21	119 $\pm$ 48	NS
NoENDO	154 $\pm$ 80	123 $\pm$ 66	142 $\pm$ 84	127 $\pm$ 59	109 $\pm$ 42	113 $\pm$ 62	95 $\pm$ 36	91 $\pm$ 48	
L_w (mm)									
ENDO	10.7 $\pm$ 1.0	11.6 $\pm$ 1.4	11.6 $\pm$ 1.3	11.4 $\pm$ 1.2	11.3 $\pm$ 1.2	11.3 $\pm$ 1.3	11.1 $\pm$ 1.2	11.3 $\pm$ 1.4	NS
NoENDO	14.6 $\pm$ 5.9	15.2 $\pm$ 5.7	15.2 $\pm$ 5.8	15.5 $\pm$ 5.9	15.5 $\pm$ 5.8	15.5 $\pm$ 5.8	15.3 $\pm$ 5.8	15.2 $\pm$ 5.9	
PRWA (%)†									
ENDO	100	44 $\pm$ 35	44 $\pm$ 28	52 $\pm$ 26	55 $\pm$ 21	57 $\pm$ 18	63 $\pm$ 25	51 $\pm$ 17	<0.02
NoENDO	100	47 $\pm$ 18	58 $\pm$ 33	39 $\pm$ 25	36 $\pm$ 26	34 $\pm$ 19	40 $\pm$ 31	35 $\pm$ 20	

Values mean $\pm$ standard deviation. NS, Not significant.

*Differences between ENDO and NoENDO groups tested by two-way analysis of variance for repeated measures.

†PRWA expressed as percentage of preischemia value.

in a significant reduction in prostacyclin release in ITA effluent.²⁸ Deendothelialization by the balloon catheter method results in selective endothelial denudation without disruption of the underlying internal elastic lamina or significant injury to the media and does not result in chronic myointimal hypercellularity.^{27, 29, 34} Also, balloon catheter endothelial

denudation has been shown to result in minimal platelet deposition on the luminal surface of canine vessels in the coronary circulation and has been shown not to affect resting coronary blood flow or the hyperemic flow response after transient coronary occlusion.³⁴ In this study, examinations of ITA conduit luminal surfaces by scanning electron mi-

Fig. 5. Effects of circumflex graft occlusion and reperfusion on myocardial contractile function in the circumflex region quantified by PRWA expressed as percentage of preischemia control values in ENDO and NoENDO groups. Values are mean $\pm$ standard error of the mean. Difference between ENDO and NoENDO groups tested by two-way analysis of variance for repeated measures.

croscopy confirmed selective endothelial denudation by the balloon catheter technique. In addition, ex vivo bioassay data confirmed disparately high and low concentrations of prostacyclin, or of a prostanoid with vasodilator activity similar to prostacyclin, in effluent of endothelialized and deendothelialized ITA conduits, respectively. Nonetheless, we were unable to detect differences in total coronary blood flow at baseline. Because of the study design, reperfusion flows in myocardial regions perfused by the two types of conduits also did not differ. We chose to use an ischemic period limited to 15 minutes, a duration of ischemia known to cause no myocardial necrosis but to result in prolonged systolic contractile dysfunction that persists for as long as 24 hours of reperfusion.³² Precise quantification of the regional contractile dysfunction and recovery in this model utilizing the load-independent preload recruitable SW relationship has been described elsewhere.³¹⁻³²

This study demonstrated an association between improved functional recovery of postischemic stunned myocardium and regional myocardial perfusion

through an endothelium-containing conduit. A clear causal relationship between increased release of endothelium-derived prostacyclin in bypass conduit effluent and accelerated myocardial recovery was not established in this study. Other work, however, has more directly demonstrated a myocardial protective function of prostacyclin. Prostacyclin and a chemically stable analog of prostacyclin, iloprost, have previously been shown to be protective in a number of animal models of myocardial ischemia and reperfusion.^{11, 12, 19-23} The administration of exogenous prostacyclin analog has been demonstrated to accelerate functional recovery of reversibly damaged, stunned myocardium after transient regional ischemia in the intact canine in a dose-dependent, time-dependent manner.²⁴ Administration of iloprost immediately before reperfusion results in significant improvement in regional functional recovery, suggesting that beneficial effects of iloprost occur during reperfusion. Functional recovery is greater when administration is begun before ischemia, however, indicating beneficial actions of iloprost both before and during coronary occlu-

sion.²⁴ The enhancement of postischemic functional recovery of stunned myocardium by prostacyclin analog has been shown not to be mediated either by alterations in transmural myocardial blood flow distribution or by preservation of myocardial adenine nucleotide content. Rather, the mechanism by which iloprost accelerates recovery of stunned myocardium seems to be mediated by inhibition of neutrophil-derived free radicals^{21, 23, 24} during reperfusion. Although they do not exert a direct superoxide scavenging action, prostacyclin and prostacyclin analogs do inhibit endothelial adhesion of neutrophils, retard neutrophil transmigration into reperfused myocardium, and block the oxidative burst of stimulated neutrophils.^{21, 23, 25}

Other studies have demonstrated that redirection of endogenous prostanoid synthesis by inhibitors of thromboxane synthetase^{12, 35, 36} to enhance prostacyclin production is also associated with improvement in myocardial functional recovery, reduction in infarct size, and suppression of reperfusion dysrhythmias. These studies have demonstrated decreased endogenous thromboxane release and increased release of 6-keto-PGF_{1 α} , a stable metabolite of prostacyclin, in coronary venous effluent from postischemic myocardial regions and have shown that the beneficial effects of redirected prostanoid synthesis are abolished by the addition of indomethacin but are unaffected by thromboxane receptor blockade.¹² This confirms that accelerated myocardial recovery is not caused by reduction of endogenous thromboxane production but rather is attributable to increased endogenous prostacyclin synthesis. In addition to playing a role as a cytoprotective and vasodilator agent, prostacyclin also has fibrinolytic and antithrombotic activity, which may also affect transmural microvascular coronary flow distribution and favorably influence postischemic myocardial performance. The exact mechanism and role of endothelium-derived prostacyclin in mediating myocellular performance and tolerance to ischemia and reperfusion injury remain to be determined.

There are several implications of this study regarding the use of bypass conduits in the coronary circulation in clinical practice. First, the potential benefits associated with use of an arterial conduit are further emphasized. In addition to the known superior long-term patency rates of ITA grafts, our findings suggest that there may be additional biologic advantages attributable to the ITA conduit. The apparent beneficial effects of ITA endothelium-

Fig. 6. Ex vivo bioassay of intraluminal release of endogenous prostanoids in excised ITA conduits in ENDO ($n = 3$) and NoENDO ($n = 2$) groups. Conduit effluent from the ENDO group induced greater relaxation of normal coronary artery smooth muscle rings (relaxation expressed as a percentage of the maximal contraction achieved by prostaglandin F_{2 α}) than did that from the NoENDO group (average 67% versus 23% relaxation). The increased smooth muscle relaxation induced by conduit effluent from the ENDO group was nearly eliminated by addition to conduit perfusate of indomethacin, a blocker of prostanoid synthesis. This indicates greater intraluminal release of endogenous prostacyclin or other endogenous vasoactive prostanoids by endothelialized conduits.

derived prostacyclin on downstream myocellular function may in part account for the early postoperative survival benefits attributed to use of an ITA conduit in patients undergoing coronary bypass grafting.³⁷ Also, additional long-term clinical benefits of ITA conduits may be derived from the fact that elevated levels of endothelium-derived prostacyclin in ITA conduit effluent may act to inhibit downstream platelet-mediated coronary vasoconstriction and also to attenuate myocardial stunning resulting from subsequent episodes of transient myocardial ischemia.

Recently, studies have indicated the feasibility of gene transfer to endothelial cells^{38, 39} and of generating segments of arterial vessels containing genetically modified cells.⁴⁰ These methods offer the possibility of favorably altering vessel wall biology to render native coronary vessels or coronary bypass conduits more resistant to thrombosis, myointimal hyperplasia, and atherosclerotic degeneration. Our data suggest the possibility that populating autologous or prosthetic bypass conduits with genetically modified endothelial cells with a high capacity for expression of specific polypeptides, such as prosta-

cyclin, might also be beneficial and should be further investigated.

Characteristics of bypass conduit vessel wall biology significantly influence downstream myocardial contractile function. The potential for engineering bypass conduits with genetically modified endothelial cells for optimal performance in the coronary circulation requires further study. ITA coronary bypass grafting in the conscious canine represents a uniquely suitable model for further investigation of endothelium-mediated myocardial events in the intact circulation.

REFERENCES

1. Singh RN, Beg RA, Kay EB. Physiological adaptability: the secret of success of the internal mammary artery graft. *Ann Thorac Surg* 1986;41:247-250.
2. Fusejima K, Takahara Y, Sudo Y, et al. Comparison of coronary hemodynamics in patients with internal mammary artery and saphenous vein coronary artery bypass grafts: a noninvasive approach using two-dimensional and Doppler echocardiography. *J Am Coll Cardiol* 1990;15:140-2.
3. deBono DP, Samani NJ, Spyt TJ, et al. Transcutaneous ultrasound measurement of blood flow in internal mammary artery compared to coronary artery grafts. *Lancet* 1992;339:379-81.
4. Sims FH. Discontinuities in the internal elastic lamina: a comparison of coronary and internal mammary arteries. *Artery* 1985;13:127-43.
5. vanSon JAM, Smedts F, Vincent JG, et al. Comparative anatomical studies of various arterial conduits for myocardial revascularization. *J THORAC CARDIOVASC SURG* 1990;99:703-7.
6. Chaikhoury A, Crawford FA, Kochel PJ, Olanoff LS, Halushka PV. Human internal mammary artery produces more prostacyclin than saphenous vein. *J THORAC CARDIOVASC SURG* 1986;92:88-91.
7. Subramanian VA, Hernandex Y, Tack-Goldman K, Grabowski EF, Weksler BB. Prostacyclin production by internal mammary artery as a factor in coronary artery bypass grafts. *Surgery* 1986;100:376-82.
8. Luscher TF, Diederich D, Siebenmann R, et al. Difference between endothelium-dependent relaxation in arterial and in venous coronary bypass grafts. *N Engl J Med* 1988;319:462-7.
9. Yang Z, Diederich D, Schneider K, et al. Endothelium-derived relaxing factor and protection against contracture induced by histamine and serotonin in the human internal mammary artery and in the saphenous vein. *Circulation* 1989;80:1041-8.
10. Vane JR, Anggard EK, Botting RM. Regulatory function of the vascular endothelium. *N Engl J Med* 1990;323:27-36.
11. Chen X, Pi XJ, Li DY, Deng HW. Prostacyclin-mediated cardioprotection of captopril and ramiprilate against lipid peroxidation in rats. In: Schror K, Sinzinger H, eds. *Prostaglandins in clinical research cardiovascular system*. New York: AR Liss, 1989:167-73.
12. Farber NE, Gross GJ. Prostaglandin redirection by thromboxane synthetase inhibition: attenuation of myocardial stunning in canine heart. *Circulation* 1990;81:369-80.
13. Balligand JL, Kelly RA, Marsden PA, Smith TW, Michel T. Control of cardiac muscle cell function by endogenous nitric oxide signaling system. *Proc Natl Acad Science U S A* 1993;90:347-51.
14. Balligand JL, Ungureanu D, Kelly RA, et al. Abnormal contractile function due to induction of nitric oxide synthesis in rat cardiac myocytes follows exposure to activated macrophage-conditioned medium. *J Clin Invest* 1993;91:2314-9.
15. Brady AJ, Poole-Wilson PA, Harding SE, Warren JB. Nitric oxide production within cardiac myocytes reduces their contractility in endotoxemia. *Am J Physiol* 1992;203 (6 Pt2):H1963-6.
16. Brady AJ, Warren JB, Poole-Wilson PA, Williams TJ, Harding SE. Nitric oxide attenuates cardiac myocyte contraction. *Am J Physiol* 1993;265 (1 Pt2):H176-82.
17. Patel VC, Yellon DM, Singh KJ, Neild GH, Woolfson RG. Inhibition of nitric oxide limits infarct size in the in situ rabbit heart. *Biochem Biophys Res Com* 1993;194:234-8.
18. Woditsch I, Schrör K. Prostacyclin rather than endogenous nitric oxide is a tissue protective factor in myocardial ischemia. *Am J Physiol* 1992;263 (5 Pt2):H1390-6.
19. Jugdutt BJ, Hutching GM, Bulkley BH, Becker LC. Dissimilar effects of prostacyclin, prostaglandin E₁ and prostaglandin E₂ on myocardial infarct size after coronary occlusion in conscious dogs. *Circ Res* 1981;49:685-700.
20. Melin JA, Becker LC. Salvage of ischemic myocardium by prostacyclin during experimental myocardial infarction. *J Am Coll Cardiol* 1983;2:279-86.
21. Simpson PJ, Mitos SE, Ventura A, et al. Prostacyclin protects ischemic-reperfused myocardium in the dog by inhibition of neutrophil activation. *Am Heart J* 1987;113:129-37.
22. Schror K, Ohlendorf R, Darius H. Beneficial effects of new carbacyclin derivative ZK 36374 in acute myocardial ischemia. *J Pharmacol Exp Ther* 1981;219:243-9.
23. Simpson PJ, Smith CB Jr, Nickelson J, Gallas M, Lucchesia BR. Canine myocardial infarct size reduction by iloprost on PGE₁: modification of neutrophil function and reduced myocardial oxygen demand [Abstract]. *Fed Proc* 1986;45:196.
24. Farber NE, Pieper GM, Thomas JP, Gross GJ. Ben-

- eficial effects of iloprost in the stunned canine myocardium. *Circ Res* 1988;62:204-15.
25. Lefer AM, Lefer DJ. Pharmacology of the endothelium in ischemia—reperfusion and circulatory shock. *Annu Rev Pharmacol Toxicol* 1993;33:71-90.
26. McCarthy PM, Schaff HV. A cost-effective technique for experimental coronary artery bypass. *J THORAC CARDIOVASC SURG* 1988;96:30-2.
27. Jorgensen RA, Dobrin PB. Balloon embolectomy catheters in small arteries. IV. Correlation of shear forces with histologic injury. *Surgery* 1983;93:798-808.
28. Johns RA, Peach MJ, Flanagan, Kron IL. Probing of the canine mammary artery damages endothelium and impairs vasodilation resulting from prostacyclin and endothelium-derived relaxing factor. *J THORAC CARDIOVASC SURG* 1989;97:252-8.
29. Barner HB, Fischer VW, Beaudet L. Effects of dilation with a balloon catheter on the endothelium of the internal thoracic artery. *J THORAC CARDIOVASC SURG* 1992;103:375-80.
30. Pearson PJ, Evora PRB, Schaff HV. Bioassay of EDRF from internal mammary arteries: implications for early and late bypass graft patency. *Ann Thorac Surg* 1992;54:1078-84.
31. Glower DD, Schaper J, Kabas JS, et al. Relation between reversal of diastolic creep and recovery of systolic function after ischemic myocardial injury in conscious dogs. *Circ Res* 1987;60:850-60.
32. Conte MS, Birinyi LK, Miyata T, et al. Efficient repopulation of denuded rabbit arteries with autologous genetically modified endothelial cells. *Circulation* 1994;23:2161-9.
33. Aarnio PTT, Harjula ALJ, Viinikka L, Merikallio EM, Mattila SP. Prostacyclin production in free versus native IMA grafts. *Ann Thorac Surg* 1988;45:390-2.
34. Bates ER, McGillem MJ, Beals JF, et al. Effect of angioplasty-induced endothelial denudation compared to medial injury on regional coronary blood flow. *Circulation* 1987;76:710-6.
35. Coker SJ. Further evidence that thromboxane exacerbates arrhythmias: effects of UK38485 during coronary artery occlusion and reperfusion in anaesthetized greyhounds. *J Mol Cell Cardiol* 1984;16:633-41.
36. Imamoto T, Terashita Z, Tanabe M, Nishikawa K, Hirata M. Protective effect of a novel thromboxane synthetase inhibitor CV1-4151 on myocardial damage due to coronary occlusion and reperfusion in the hearts of anesthetized dogs. *J Cardiovasc Pharmacol* 1986;8:832-9.
37. Grover FL, Johnson RR, Marshall G, Hammermeister KE. Impact of mammary grafts on coronary bypass operative mortality and morbidity. *Ann Thorac Surg* 1994;57:559-68.
38. Chen S, Wilson JM, Muller DW. Adenovirus-mediated gene transfer of soluble vascular cell adhesion molecule to porcine interposition vein grafts. *Circulation* 1994;89:1922-8.
39. Willard JE, Landau C, Glamann DB, et al. Genetic modification of the vessel wall: comparison of surgical and catheter-based techniques for delivery of recombinant adenovirus. *Circulation* 1994;89:2190-7.
40. Glower DD, Spratt JA, Kabas JS, Davis JW, Rankin JS. Quantification of regional myocardial dysfunction after acute ischemic injury. *Am J Physiol* 1988;255 (1 Pt2):H85-93.