Repeated Dipyridamole Administration Enhances Collateral-Dependent Flow and Regional Function During Exercise

A Role for Adenosine

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Two main hypotheses concerning the mechanisms responsible for coronary collateral growth suggest the involvement of chemical or mechanical factors. Since we recently demonstrated that the development of the coronary collateral circulation is not closely related to the extent or duration of myocardial ischemia, we hypothesized that chronic repeated vasodilation and increased myocardial blood flow using dipyridamole would enhance collateral development in miniswine with an ameroid-occluded left circumflex coronary artery (LCx). Two days after surgical instrumentation, the animals received dipyridamole (n=9), diltiazem as an adenosine-independent vasodilator (n=8), or control vehicle (n=7) 90 minutes per day, 5 days per week for 8 weeks. At 5 and 8 weeks, transmural blood flow and systolic wall thickening were measured during infusion of dipyridamole, diltiazem, or vehicle. Transmural blood flow increased similarly in the LCx and nonoccluded regions at 30 and 60 minutes during infusion of either vasodilator. Thus, we believe that similar mechanical stimulation resulted from dipyridamole and diltiazem infusion. There was no change in blood flow during administration of the vehicle. Systolic wall thickening in the collateral-dependent region was not altered by infusion of dipyridamole, diltiazem, or vehicle. Therefore, both vasodilators increased blood flow without eliciting ischemia. After 8 weeks of repeated treatment with each pharmacological agent, at least 24 hours after the last drug infusion, near maximal physiological capacity of the coronary collateral vessels was assessed during treadmill running (=240 beats per minute). Transmural myocardial blood flow ratios, expressed as flow in the LCx divided by flow in the nonoccluded region of the left ventricle, were similar at rest for animals treated with dipyridamole (0.90±0.03), diltiazem (0.97±0.05), and control vehicle (0.89±0.02). However, collateral-dependent myocardial blood flow during exercise was greater (P<.05) in the dipyridamole-treated animals (0.78±0.04) than in either diltiazem-treated (0.63±0.09) or vehicle-treated (0.62±0.02) animals. LCx systolic wall thickening at rest was similar in animals treated with dipyridamole (44.4±6.3%), diltiazem (42.2±3.0%), and control vehicle (38.1±2.8%). During exercise, however, myocardial function in the collateral-dependent region was greater (P<.05) in the dipyridamole-treated (39.2±5.2%) compared with diltiazem-treated (23.9±4.0%) and vehicle-treated (26.9±2.9%) animals. Our results suggest that enhanced collateral-dependent blood flow and function in dipyridamole-treated animals likely result from a direct effect of adenosine and/or from an adenosine-potentiated vascular response to repeated mechanical stress rather than a response simply related to mechanical stimulation. (Circulation Research 1993;73:503-513)

KEY WORDS • coronary collaterals • myocardial blood flow • myocardial function • dipyridamole • adenosine • angiogenesis

The protective nature of coronary collateral vessels is widely accepted, but the precise mechanism(s) responsible for their growth and development is not fully understood. Two main hypotheses that have been advanced suggest the involvement of chemical or mechanical factors in the formation and/or remodeling of this vasculature. Previously, we demonstrated that exercise training improves coronary collateral flow and lessens ischemic dysfunction in collateral-dependent myocardium. Since the acute response to exercise involves increased myocardial blood flow and regional ischemia in the collateralized zone, repeated exposure to both chemical and mechanical stimuli potentially could have contributed to collateral growth during physical training. We recently investigated the contribution of myocardial ischemia to collateral flow development in miniswine during gradual occlusion of the left circumflex coronary artery (LCx) using an ameroid occluder. Although chronic β-adrenoceptor blockade virtually eliminated myocardial ischemia in these sedentary animals, collateral-dependent blood flow and regional function during moderately severe treadmill exercise were similar to the values found in unblocked animals. Our data suggest that growth and

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development of the coronary collateral circulation are not closely related to the extent or duration of myocardial ischemia in this model. Therefore, it is possible that mechanical stimulation, rather than the process of ischemia, is a major determinant of coronary collateral development in miniswine.

Some investigators have suggested that, during gradual occlusion of a coronary artery, mechanical factors such as circumferential, radial, longitudinal, and/or shear stresses act on the walls of developing collateral vessels to enhance the growth of this vasculature. All of these mechanical forces can be augmented by elevating coronary blood flow and/or the intracollateral pressure. Evidence implicating mechanical factors in promoting collateral growth has been provided by observing regression of newly formed vessels when exposure to increased flow is discontinued.

Experimental paradigms used previously to elevate coronary flow and increase collateral vascular wall stress include repeated intermittent coronary artery occlusions, daily exercise sessions, and repeated administration of vasodilatory drugs. Although all three procedures improved collateral-dependent blood flow, several considerations must be addressed regarding the respective methodologies. First, repeated coronary artery occlusions and exercise dramatically increase both myocardial ischemia and vascular wall stress in the collateral-dependent myocardium. Therefore, it is difficult to determine the relative contribution of mechanical and chemical stimuli to collateral development using these experimental interventions. Second, previous studies evaluating the effects of repeated administration of vasodilatory drugs neither directly assessed the maximal blood flow capacity of the collateral vasculature during a physiological stimulus nor measured myocardial function in the collateralized region to confirm the physiological relevance of any improvements in collateral blood flow. Moreover, the possibility that increased collateral-dependent blood flow may have resulted directly from chemical factors associated with the mechanism of action of each respective vasodilator has not been adequately addressed. Consequently, although some results suggest that collateral vessel function may be enhanced after repeated exposure to vasodilatory drugs, conclusive evidence has not been provided, and any direct contributions of the drug used to elicit vasodilation have not been determined.

Most investigations concerned with enhancing coronary collateral circulation development by repeated administration of a vasodilator have used dipyridamole. Dipyridamole provides mechanical stimulation by eliciting vasodilation and elevating coronary blood flow to the native and collateral regions in our model. Dipyridamole works by inhibiting myocardial cell and erythrocyte uptake of adenosine, thereby increasing the interstitial concentration of this purine to cause vasodilation. However, because adenosine also causes endothelial cell proliferation and migration in vitro, promotes angiogenesis in the chick chorioallantoic membrane, and increases myocardial capillary density in rabbits, a direct mitogenic effect of adenosine cannot be excluded. Therefore, like intermittent coronary artery occlusions and daily exercise, separation of the mechanical from chemical contributions to collateral development is difficult in studies using repeated administration of dipyridamole.

The purpose of the present study was to evaluate the effects of repeated mechanical stimulation by dipyridamole on development of the coronary collateral circulation and hence the adequacy of collateral perfusion during and after gradual occlusion of the LCx using an ameroid occluder. Since dipyridamole elevates interstitial concentrations of adenosine that may directly influence vascular development by a chemical mechanism, we also determined the effects of an adenosine-independent vasodilator, diltiazem, on development of the coronary collateral circulation. Diltiazem is a slow-channel calcium antagonist capable of increasing blood flow to both native and collateral-dependent myocardium. We hypothesized that chronic repeated mechanical stimulation using each vasodilator increases coronary collateral growth and development such that collateral-dependent flow during treadmill exercise is greater compared with flow in a vehicle-treated control group. Furthermore, the physiological meaning of any increase in collateral-dependent blood flow would be confirmed by measuring the extent of myocardial ischemic dysfunction during treadmill running. Finally, since previous investigations suggest that long-term administration of dipyridamole may have an angiogenic effect documented as an increase in myocardial capillary density, we hypothesized that improved collateral blood flow in dipyridamole-treated animals is accompanied by enhanced growth of these microcirculatory vessels.

Materials and Methods

Surgical and experimental protocols used in this investigation were approved by the Animal Use and Care Committee at the University of California, Davis. During a 2- to 4-week period before surgery, miniswine were familiarized with human handling, transport procedures, standing, walking, and running on the motorized treadmill, and wearing a protective jacket. The pigs were housed individually in pens and fed twice daily.

Surgical Procedure

One day before surgery, the pigs were given 400 mg sulfamethoxazole and 80 mg trimethoprim orally (Tribrisson) and then were fasted overnight. Immediately before surgery, the animals were sedated with ketamine (25 mg/kg IM) and atropine (0.05 mg/kg IM) and were intubated after relaxation by mask induction with 1% to 3% halothane. During the aseptic surgical procedure, anesthesia was maintained with 1% to 2% isoflurane. A left lateral thoracotomy was performed through the fifth intercostal space, and the animals were instrumented with left atrial, pulmonary artery, and proximal descending aortic silastic catheters, a high-fidelity left ventricular pressure transducer (model P 6.5, Konigsberg), and four epicardial recording electrodes. A 1.0- to 1.5-cm segment of the proximal LCx was dissected free from surrounding tissue, and a metal-encased ameroid constrictor (model K-G, Ulrich, Montreal, Quebec, Canada) was placed around the vessel. The size of the constrictor (2.0- to 3.0-mm lumen) was determined at surgery so that the lumen would provide a close but nonocclusive fit around the artery. The left anterior descending coronary artery (LAD) also was dissected free, and a Doppler flow probe (2.5 mm,
Crystal Biotech, Holliston, Mass) was placed around the vessel. Sonomicrometer dimension gauges (5 MHz, 2.5 mm in diameter, J.W. Inc, San Diego, Calif) were placed across the left ventricular free wall for measurement of wall thickness (Triton Technology, San Diego, Calif). The subendocardial crystal of the pair was held at the tip of a Teflon tube (outer diameter, 1.75 mm) and advanced diagonally through the myocardium in a tract created by an 18-gauge metal cylinder. The second crystal of the pair was sewn to the epicardial surface of the heart with an attached Dacron patch. Two sets of crystals were placed 1 to 2 cm below the LCx, distal to the atheromatous constrictor. These were used to assess regional myocardial function in the collateral-dependent region. Accurate placement of the sonomicrometer dimension gauges was confirmed by the presence of decreased wall thickening or frank wall thinning in the LCx region in response to a brief (10- to 20-second) occlusion of the LCx at the level of the constrictor. All catheters and wires were exteriorized on the pig’s back. After surgery, each animal was refitted with a custom-made jacket (Alice King Chatham, Los Angeles, Calif, or Fabric Expressions, Seattle, Wash) to protect catheters and wounds from injury.

The health status of the miniswine was monitored at least twice daily, 7 days per week. Trubrisson was administered orally each day throughout the protocol. Catheters were flushed, exit sites were cleaned, and rectal temperatures were obtained at least three times per week. All animals were weighed once per week.

**Hemodynamic Measurements**

Systolic, diastolic, and mean arterial blood pressure levels, heart rate (HR), left ventricular pressure, left ventricular dp/dt at a developed pressure of 30 or 40 mm Hg (dp/dt at DP 30 or DP 40), left ventricular end-diastolic pressure (LVEDP), ECG, and signals obtained from the sonomicrometer dimension gauges were monitored and recorded on a Gould TA 4000, Cleveland, Ohio. The heart rate times systolic blood pressure product (RPP) was used to estimate myocardial oxygen demand, and dp/dt at a developed pressure was used to estimate global myocardial function, since it is more independent of changes in preload and afterload than maximal dp/dt.31 Hemodynamic variables measured immediately before and after each particular blood flow determination using microspheres were similar and therefore were combined.

Regional myocardial blood flow was measured by injecting ≈3x10⁶ radiolabeled microspheres (15 μm, New England Nuclear, Boston, Mass) into the left atrium. Spheres were suspended in 10% dextran and 0.01% Tween 80 solvent and were agitated with a vortex mixer before injection. Radioisotopes used included scandium-46, niobium-95, indium-114, ruthenium-103, tin-113, chromium-51, cerium-141, and stronitum-85. Reference blood samples were withdrawn (model 351, Sage, Cambridge, Mass) from the aortic catheter at 7.5 mL/min for 120 seconds. Regional myocardial and renal blood flows were calculated according to the method of Heymann et al.32 Myocardial and reference blood sample were analyzed for the quantity and energy level of gamma radiation using a germanium detector (Micrad Inc, Knoxville, Tenn). Regional myocardial blood flow (Qm) was computed using the formula Qm=Q, - Cm/Cr,

where Q, is the reference flow rate (in milliliters per minute), Cm indicates counts per minute from the myocardial tissue sample, and Cr indicates counts per minute from the reference blood sample. Each blood flow measurement was normalized for the weight of the myocardial tissue sample. Control blood flows in the LAD and right coronary artery (RCA) regions were similar and therefore were combined for comparison with blood flow in the LCx perfusion territory, ie, the collateral-dependent region. To account for variable flows between pigs, transmural blood flow ratios were obtained by dividing transmural flow in the collateral region by transmural flow in the control region. Blood flows in the cortex and medulla of each kidney also were determined to document adequate mixing of the microspheres. Mixing was confirmed if the difference in blood flow between the right and left kidney was <15%.

**Regional Myocardial Function**

Regional myocardial function was assessed with sonomicrometer dimension gauges. LCx systolic wall thickening was measured over the ejection phase of systole. The transit time of sound traveling between the ultrasonic crystals was measured as the distance between the crystals. In pigs with functional Konigsberg transducers, the ejection phase was defined as the time from the onset of rise of dp/dt to 20 milliseconds before peak negative dp/dt.33 In animals without functional Konigsberg transducers, the timing of systole was determined using the peak of the S wave and the end of the T wave of the ECG. It has been shown previously that no difference exists between dp/dt or the ST interval when calculating the systolic time interval.34 These data are expressed as percent wall thickening, calculated as end-systolic wall thickness minus end-diastolic wall thickness divided by end-diastolic wall thickness times 100. All hemodynamic and regional function calculations were determined over 5 and 10 consecutive cardiac cycles during rest and exercise, respectively. LCx systolic wall thickening measured immediately before and after each particular blood flow determination using microspheres was similar and therefore was combined.

**Experimental Protocols**

After surgery, the animals were separated into a group that received either dipyridamole (28.4±2.0 μg/kg per minute, Chemischpharmazeutische Fabrik, Biberach, Germany), diltiazem (129±8.0 μg/kg per minute, Marion Merrell-Dow, Kansas City, Mo), or the control vehicle, tartaric acid (14.0±0.0 μg/kg per minute, Sigma Chemical Co, St Louis, Mo). Tartaric acid was chosen as the vehicle since it was used for solubilizing both dipyridamole and diltiazem. The first drug administration was performed 2 days after surgery, for ≈30 to 60 minutes. Thereafter, infusion of each respective drug was performed 90±1 minutes per day, 5 days per week for 34±1 days.

**Dipyridamole, diltiazem, or tartaric acid infusion.** Animals received dipyridamole (body weight at surgery, 29±3 kg; n=9) or diltiazem (body weight at surgery, 26±2 kg; n=8) at a concentration and rate sufficient to elicit the greatest equivalent increase in LAD blood flow velocity with both drugs with no attenuation of LCx systolic wall thickening. Systolic, mean, and diastolic arterial pressures were monitored during every drug...
infusion, and LCx systolic wall thickening was monitored at least three times per week. The rate of each drug infusion was titrated such that mean arterial pressure did not decrease >20 mm Hg from baseline values, and diastolic blood pressure was not reduced below 50 mm Hg. Seven animals (body weight at surgery, 26±2 kg) served as vehicle controls, and the rate of infusion was calculated from the average amount used to solubilize dipyridamole and diltiazem.

Five and 8 weeks after surgery, hemodynamic variables, LAD blood flow velocity, LCx systolic wall thickening, and blood gases (model ABL3, Radiometer, Copenhagen, Denmark) were obtained at rest and during dipyridamole, diltiazem, or vehicle infusion at 30, 60, and 90 minutes in all animals. In a subset of animals, myocardial blood flow was obtained at rest and at 30 and 60 minutes of each respective infusion. Blood flow was not obtained at 90 minutes. The 5- and 8-week responses to each respective drug infusion were similar, and the data were combined. This protocol was performed to document the hemodynamic and hematologic responses to each drug.

Treadmill exercise. Five and 8 weeks after surgery, hemodynamic variables, myocardial blood flow, LCx systolic wall thickening, blood gases, and lactate concentrations (model 23L, Yellow Springs Instrument Co, Yellow Springs, Ohio) were obtained in all animals while they stood quietly on the treadmill and again during treadmill exercise. Exercise consisted of treadmill running at 2 mph and 2% grade for 2 minutes and at 3 mph and 2% grade for 3 minutes, followed by adjustments in speed and grade to achieve and maintain an HR of 240 beats per minute for ≈4 minutes. This exercise intensity was chosen since it elicited near-maximal physiological capacity of the collateral vessels and can be maintained as a steady state during the determination of blood flow.6 Neither pharmacological agent was administered 24 hours before treadmill running. This protocol was performed to assess the effects of repeated administration of each respective drug on the near-maximal functional capacity of the collateral-dependent myocardium. The responses to treadmill exercise were similar for each group at 5 and 8 weeks; therefore, the data were combined.

Measurement of dipyridamole. Plasma concentrations of dipyridamole were obtained from five animals before, at 30 and 90 minutes of drug administration and at 60, 120, and 180 minutes after the infusion. Plasma dipyridamole was measured using high-performance liquid chromatographic fluorescence.6 Brieﬂy, 2-mL arterial blood samples were collected, centrifuged at 4000 rpm for 20 minutes at 4°C, and stored at −70°C. Next, low and high internal standards were added to calibration standards or plasma samples and vortexed for 30 seconds. After the addition of 3 mL anhydrous ethyl ether, each tube was vortexed for 30 seconds, followed by centrifugation for 5 minutes at 2000 rpm. Tubes then were placed in granulated dry ice to freeze the aqueous phase. The organic phase was evaporated under puriﬁed nitrogen at room temperature. After evaporation of the organic phase, ether was added to each tube and vortexed, and the tubes were evaporated as described. The remaining residue was reconstituted by the addition of 50 µL methanol for low-range calibration standards and 100 µL methanol for high-range calibration standards. Samples were vortexed, and 25 nL was injected for chromatographic separation. The coefﬁcient of variation was 4.5%, and the limit of detection was 5 ng/mL. This protocol was performed to assess the effects of dipyridamole infusion on plasma concentrations of this pharmacological agent.

Measurement of prostaglandins. Prostaglandin (PG) E2 and 6-keto-PGF1α, the stable metabolite of PGF1α, were measured before and during dipyridamole infusion in three pigs. Animals were sedated and intubated as described above. Anesthesia was maintained using sodium thiamylal (20 mg/kg IV). An 8F angiographic catheter was inserted into the right jugular vein and passed retrogradely into the coronary sinus. Placement was veriﬁed using ﬂuoroscopy and blood gases. After approximately 180 minutes, to allow time for endogenous PG release due to surgical trauma to subside, dipyridamole (30 µg/kg per minute) was infused into the left jugular vein for 90 minutes. Coronary sinus blood samples were obtained before infusion and at 30, 60, and 90 minutes for the measurement of PGs. Two mL of blood was collected in syringes prepared previously (5 µL heparin and 5 µL ibuprofen) and placed in tubes containing 80 µL EDTA and 10 µL ibuprofen. Next, samples were centrifuged at 4000 rpm for 20 minutes at 4°C and stored at −70°C. PGs were extracted from plasma by adsorption onto C-18 reverse-phase cartridges (Sep-Pak, Fisher, San Francisco, Calif) at pH 2 to 3. An enzyme immunoassay was performed on extracted plasma to determine the absolute concentration of immunoreactive PGE2 and immunoreactive 6-keto-PGF1α using a commercially available kit (Cayman Chemical, Ann Arbor, Mich). Specificity for the immunoreactive PGE2 assay was 100% for immunoreactive PGE2, 9.2% for 15-keto-PGE2, 5% for PGE1, and <0.2% for all other PGs. Specificity for the immunoreactive 6-keto PGF1α assay was 100% for immunoreactive 6-keto PGF1α, 8.7% for 2,3-dinor-6-keto-PGF1α, 2.1% for PGF1α, 0.92% for PGE2, and <0.1% for all other PGs. PG concentrations were estimated by comparison with a standard curve. The limit of detection was 4 pg/mL for PGE2 and 6-keto-PGF1α. The coefﬁcient of variation, expressed as the standard deviation of the difference between triplicate determinations relative to the total mean value for standard curve points, was 11%. Plasma samples were analyzed in duplicate. This protocol was performed to assess the effects of dipyridamole infusion on coronary sinus concentrations of PGE2 and 6-keto-PGF1α.

Euthanization and Postmortem Procedures

A condition for inclusion in this study was complete closure of the ameroid constrictor. To document closure, an angiogram of the left coronary artery system was performed (Siremobil 4U, Siemens, Iselin, NJ; Fluoro 100 Series Digital Image Processor, Eigen, Nevada City, Calif). The heart then was arrested in diastole using saturated KCl. After rapidly excising the heart from the chest, the aortic root was perfused retrogradely with 1 L phosphate buffer and 2 L phosphate-buffered glutaraldehyde. Catheters were placed in the LAD and RCA and in the LCx distal to the ameroid occluder. Through these catheters, phosphate-buffered glutaraldehyde and different colored dyes (Sigma) were perfused under physiological pressure to delineate regions of myocardium sub-
served by the LAD and RCA (control regions) and LCx (collateral-dependent region). Finally, the heart was suspended in buffered glutaraldehyde for at least 24 hours before sectioning. The myocardium was cut into five transverse rings (1 to 1.5 cm thick) along the hoop axis from base to apex. Tissue from the two most basal rings (1 and 2) was used in the analysis of myocardial blood flow, since these regions correspond to the center of the collateralized bed.\textsuperscript{7,7} The central zone of each perfusion bed was divided transmurally into three pieces of approximately equal thickness corresponding to the endocardial, midmyocardial, and epicardial layers. Therefore, six tissue sections from the LCx, LAD, and RCA were used in the blood flow analysis (ie, three tissue sections from each region two rings). The mean weights of tissue from rings 1 and 2 of the collateralized (LCx) region were 1.32±0.20, 0.72±0.07, and 1.06±0.12 g for the epicardial, midmyocardial, and endocardial layers, respectively. In the control region, tissue weights were 1.22±0.14, 0.80±0.06, and 1.07±0.08 g for the epicardial, midmyocardial, and endocardial layers, respectively.

After measurement of blood flow, the same tissue samples were used for determining percent infarction and capillary density. The extent of infarction in the collateralized region was identified by histological analysis and a quantitative morphometric point-counting technique.\textsuperscript{38} Epicardial, midmyocardial, and endocardial tissue sections were embedded in paraffin, cut (4 μm) using a microtome, and stained with Masson’s trichrome stain to identify fibrous necrotic tissue.\textsuperscript{39} Each slide was point-counted, and the percentage of necrotic tissue was measured. A sheet containing a series of 100×100-μm grids was projected onto the field of a microscope (Leitz Wetzlar, Germany), overlying the histological slide. The number of test grid intersections lying on necrotic tissue was counted and recorded. The formula for calculating volume ratios using the point counting method was \( V_t/V_{tissue} = P/P_{tissue} \), where \( V_t \) is the volume of necrotic tissue, \( V_{tissue} \) is the volume of the total tissue sample, \( P \) is the number of points lying over necrotic tissue, and \( P_{tissue} \) is the total number of points lying over the total tissue sample.\textsuperscript{40}

Capillary density (vessels per square millimeter) was measured in 2-μm-thick hematoxylin-eosin-stained plastic embedded sections that had been cut perpendicularly to the fiber orientation (JB-4 microtome, Dupont, Sorvall).\textsuperscript{39} Perfusion fixation facilitated the counting of capillaries, since they were fixed in an open position. Capillary density was determined by counting the total number of vessels with diameters \( \leq 10 \mu m \)\textsuperscript{30,41} located within a series of test grids (100×100 μm). The analysis comprised at least 20 fields, in two blocks of tissue, from rings 1 and 2 for percent infarction and capillary density.

**Statistical Analysis**

Data are expressed as group mean±SEM. A two-way analysis of variance (ANOVA) with repeated measures over time was used to examine the hemodynamic and hematologic variables, transmural blood flow, and regional myocardial function responses to infusion of dipyramidole, diltiazem, and vehicle. Post hoc tests (Bonferroni correction for multiple comparisons) were performed when significant interactions between group and time were obtained \((P<0.05)\).\textsuperscript{42} Comparison of hemodynamic variables, transmural blood flow ratios, regional myocardial function, blood lactate, capillary density, and infarction were made using a two-way ANOVA. When significant main effects were obtained, post hoc tests were performed as described above.

**Results**

Percent infarction in the collateralized region of dipyramidole-treated (9±1%, 11±2%, and 11±2%), diltiazem-treated (12±2%, 10±1%, and 10±1%), and vehicle-treated control (9±2%, 6±1%, and 10±2%) animals did not differ between groups or within the epicardial, midmyocardial, and endocardial layers, respectively.

**Responses to Dipyramidole, Diltiazem, and Control Vehicle**

**Plasma dipyramidole.** Samples used for determining plasma concentrations of dipyramidole were obtained from five animals. Each animal received dipyramidole at least once before the day samples were obtained. Dipyramidole averaged 4±4, 178±97, and 264±114 ng/mL before infusion and after 30 and 90 minutes of infusion, respectively. Samples measured 60, 120, and 180 minutes after termination of drug administration averaged 131±52, 23±5, and 12±7 ng/mL, respectively.**

**Plasma prostaglandins.** PGE\(_2\) and 6-keto-PGF\(_{1α}\) were measured in plasma obtained from the coronary sinus during administration of dipyramidole. PGE\(_2\) averaged 44.3±6.3, 43.3±8.2, 57.7±13.7, and 42.3±21.5 pg/mL, and 6-keto-PGF\(_{1α}\) averaged 75.0±14.2, 84.0±11.4, 77.3±17.4, and 86.3±21.5 pg/mL before drug infusion (control) and after 30, 60, and 90 minutes of infusion, respectively. There were no differences between the control or infusion periods for either PG.

**Hemodynamic and hematological variables.** Administration of dipyramidole and diltiazem reduced systolic, diastolic, and mean arterial pressures, increased HR, and did not alter RPP, dP/dt at DP 30, or LVEDP throughout the 90-minute infusion period (Table 1). Systolic blood pressure and RPP were lower in dipyram- dolamole-treated than diltiazem-treated animals. Mean and diastolic arterial pressure were similar in the two treated groups. Hemodynamic responses were not altered by administration of the vehicle. Systolic, diastolic, and mean arterial pressures were greater, while HR was lower, at 30, 60, and 90 minutes in the vehicle-treated compared with the dipyramidole-treated and diltiazem-treated animals. Except for PCO\(_2\), which was lower 30 minutes after diltiazem infusion, each respective drug infusion did not alter arterial pH, PO\(_2\), PCO\(_2\), HCO\(_3^-\), or base excess.

**Myocardial blood flow and LCx systolic wall thickening.** Compared with control blood flow, peak LAD blood flow velocity at 30, 60, and 90 minutes was elevated by 85±12%, 83±15%, and 84±13%, respectively, during dipyramidole infusion and by 81±6%, 77±13%, and 85±13% during diltiazem administration. Blood flow velocity was unchanged in response to the vehicle (1±9%, -15±18%, and -4±8%). There were no differences between the two treated groups. However, peak LAD blood flow velocity was significantly elevated at each time point compared with the vehicle-treated control animals.
Myocardial blood flow measurements at 5 and 8 weeks before and at 30 and 60 minutes of diprydamole, diltiazem, or vehicle infusion were similar; therefore, the data were combined (Fig 1). Transmural blood flow increased similarly in the collateral and control regions at 30 and 60 minutes during infusion of diprydamole and diltiazem. There was no change in blood flow to either region during infusion of the vehicle (Fig 1). LCx systolic wall thickening was not altered from preinfusion values in all three groups (Table 1). Systolic, diastolic, and mean arterial pressures, HR, RPP, dp/dt at DP 30, LVEDP, and LCx systolic wall thickening in each subset of animals used for blood flow determinations were not different from the values obtained using all animals (Table 1).

Responses to Exercise  
Hemodynamic variables. The increase in systolic, diastolic, and mean arterial pressures, HR, RPP, and dp/dt at DP 40 during measurement of myocardial blood flow at exercise was not different among the three groups of animals (Fig 2). Also, blood lactate concentration increased similarly from rest to exercise among the three groups (from 0.9±0.1 to 4.8±1.3 mM, from 1.0±0.1 to 4.3±0.5 mM, and from 0.8±0.1 to 5.1±1.0 mM for the diprydamole-, diltiazem-, and vehicle-treated animals, respectively). Since treadmill speed and grade, time to reach 240 beats per minute, and arterial blood lactate levels were similar among all groups, we believe that no residual drug effects were present during determination of myocardial blood flow.

Myocardial blood flow and regional function. Transmural blood flow ratios, expressed as collateral region flow divided by control region flow, which normalizes for interanimal variability, are shown in Fig 3. Although

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**Table 1. Hemodynamic Responses to Diprydamole, Diltiazem, or Vehicle Administration**

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<tr>
<th>Diprydamole (min)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>HR (bpm)</th>
<th>RPP (×10⁻⁴)</th>
<th>dp/dt at DP 30 (mm Hg/s)</th>
<th>Systolic Wth (%)</th>
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<td>0</td>
<td>121±4</td>
<td>78±4</td>
<td>95±3</td>
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<td>30</td>
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<td>57±2*</td>
<td>74±2*</td>
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<th>Diltiazem (min)</th>
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<th>DBP (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>HR (bpm)</th>
<th>RPP (×10⁻⁴)</th>
<th>dp/dt at DP 30 (mm Hg/s)</th>
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<th>DBP (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>HR (bpm)</th>
<th>RPP (×10⁻⁴)</th>
<th>dp/dt at DP 30 (mm Hg/s)</th>
<th>Systolic Wth (%)</th>
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SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; LVEDP, left ventricular end-diastolic pressure; HR, heart rate; bpm, beats per minute; RPP, heart rate times systolic blood pressure product; DP 30, developed pressure of 30 mm Hg; Wth, left circumflex systolic wall thickening. Values are mean±SEM (diprydamole, n=9; diltiazem, n=8; vehicle, n=7). For LVEDP and dp/dt at DP 30, n=4 for each group.

*P<.05 vs value at 0 minutes.
†P<.05 vs corresponding values for diltiazem and vehicle.
‡P<.05 vs corresponding value for diltiazem.
§P<.05 vs corresponding values for diprydamole and diltiazem.
resting transmural flow ratios were similar in each group, the decrease during exercise was less for dipyridamole-treated compared with diltiazem-treated and vehicle-treated control animals. Transmural blood flow ratios comparing diltiazem-treated and vehicle-treated control animals were not different. At rest, LCx systolic wall thickening was not different among groups (Fig 3). However, like the blood flow response during exercise, myocardial function in the collateral region was reduced less in the dipyridamole-treated compared with both the diltiazem-treated and vehicle-treated control groups. LCx systolic wall thickening was similar comparing diltiazem-treated and vehicle-treated control animals.

**Capillary Density**

There were no differences in capillary density among the three groups or between regions within each group (Table 2).

**Discussion**

The results of this study refute our original hypothesis that chronic repeated mechanical stimulation alone is capable of increasing coronary collateral growth and development. When near-maximal physiological capacity of the collateral vessels was assessed during exercise, we found that collateral-dependent blood flow was greater in dipyridamole-treated compared with diltiazem-treated and vehicle-treated animals. Therefore, we believe that increased collateral-dependent blood flow resulted from a direct effect of adenosine and/or from an adenosine-potentiated vascular response to repeated mechanical stress. Moreover, increased blood flow to the collateralized myocardium in dipyridamole-treated animals was physiologically meaningful, since exercise-induced ischemic dysfunction was reduced. Finally, we observed no alterations in transmural myocardial capillary density among the three groups of miniswine. Thus, we believe that increased collateral flow in the dipyridamole-treated animals resulted from remodeling of preexisting anastomotic channels rather than from formation of new vessels, since our index of angiogen-
esis (ie, capillary density) was not altered among the experimental groups.

Two general mechanisms thought to be responsible for growth and development of coronary collateral vessels are mechanical forces and the process of myocardial ischemia. We recently evaluated the contribution of myocardial ischemia to collateral flow development in miniswine. In that study, we examined whether chronically β-adrenergceptor–blocked animals developed collateral vessels to the same extent as unblocked animals in response to gradual occlusion of the LCx using an ameroid constrictor. Virtual elimination of myocardial ischemia in β-adrenergceptor–blocked animals was documented by continuously recording myocardial oxygen demand and myocardial function using biotelemetry.8-10 Eight weeks after surgery, all animals performed treadmill exercise in the absence of β-adrenergceptor blockade to assess the near-maximal physiological capacity of the coronary collateral vasculature. Blood flow and regional function in the collateral-dependent myocardium were similar in unblocked and β-blocked animals. These results indicated that severe and prolonged myocardial ischemia are not required for coronary collateral growth and development in our model. Therefore, we sought to determine the contribution of mechanical mechanisms to collateral vessel development.

There is a potential for mechanical forces to contribute to vascular growth during the process of vessel occlusion since increased blood pressure and flow velocity differences during development occur across the collateral circulation.11 In vitro studies indicate that physical forces such as repetitive mechanical stretching can modulate cellular function and induce proliferation.12-14 Likewise, turbulent flow applied to cultures of endothelial cells increases thymidine incorporation and DNA synthesis.15 Although the precise mechanical forces that contribute to collateral growth in vivo are difficult to measure, several possibilities have been suggested. For instance, tangential wall stress is markedly increased at the time of maximal collateral growth.16 In addition, theoretical analysis predicts that, after ischemia has altered the cellular structure of a vessel, circumferential, radial, longitudinal, and shear stresses are mechanical forces that may be capable of eliciting cellular proliferation.4 Experimental paradigms previously used to elevate coronary flow and increase collateral vascular wall stress include repeated intermittent coronary artery occlusions,17-20 daily exercise sessions,2 and repeated administration of vasodilatory drugs.21-23 Although these procedures improved collateral-dependent blood flow, the methodologies used in each study involve a combination of potential angiogenic stimuli. First, repeated occlusion of a coronary artery elicits pressure differences between the occluded and nonoccluded regions of the myocardium, resulting in maximal vasodilation and severe ischemia in the collateral-dependent region. These increased pressure gradients augment blood flow through preformed collateral channels during each occlusion and contribute to increased blood flow velocity and vessel wall stress in the collateral-dependent region immediately after each occlusion.24-27 Second, the acute response to exercise involves increases in blood flow velocity and intravascular pressure in the native and collateral circulation and severe myocardial ischemia in the collateral-dependent region.2 Therefore, separating the contribution of mechanical forces and factors associated with the process of ischemia to coronary collateral growth and development is difficult in studies using repeated coronary occlusions and long-term exercise. Third, previous studies using repeated administration of vasodilators to enhance collateral vessel development did not assess the possible direct mitogenic effects of the pharmacological agent used. For instance, dipyridamole increases the concentration of adenosine in the coronary vasculature.21 Since evidence exists demonstrating that, in addition to its vasodilator properties, adenosine may be angiogenic and potentiate the vascular response to other mitogenic stimuli,22,24,25 the contribution of this factor to collateral-dependent flow development may be important. Moreover, a role for adenosine in contributing to increased collateral-dependent blood flow development observed after repeated exercise sessions or coronary occlusions cannot be discounted, since these interventions increase adenosine concentrations as well.49 Therefore, like intermittent coronary artery occlusions and daily exercise, separating mechanical from chemical contributions to collateral development is difficult in studies using repeated administration of substances such as dipyridamole. Additionally, previous studies using repeated administration of a vasodilator did not directly measure maximal blood flow capacity of the collateral vasculature during physiological stimulation and did not measure myocardial function in the collateralized region to confirm the physiological relevance of any improvements in collateral blood flow.13-19 Therefore, ambiguity exists regarding the ability of substances such as dipyridamole to elicit increases in coronary collateral blood flow that are physiologically meaningful.

In light of these considerations, we examined the contribution of repeated mechanical stimulation to collateral growth and development using an adenosine-dependent (ie, dipyridamole) and an adenosine-inde-
ependent (ie, diltiazem) vasodilator. We felt that the acute response to vasodilation would enhance both circumferential and longitudinal vessel wall stress since, despite reduced intraluminal pressure, these stresses are augmented because vessel radius is increased. Furthermore, shear stress is increased acutely by dipyridamole and diltiazem because blood flow velocity is elevated through dilated collateral channels. We chose to use dipyridamole, since data from previous investigations, although inconclusive, suggested that it could promote collateral flow development. Diltiazem was chosen as our adenosine-independent vasodilator since preliminary data from our laboratory indicated that blood flow elevations to native and collateral-dependent myocardium were similar to those obtained using dipyridamole. By measuring LCx systolic wall thickening at least three times per week in each animal, we also verified that regional function was not compromised and hence ischemia did not occur during administration of either vasodilator.

The miniswine was selected as an experimental model because the anatomy and physiology of its coronary collateral circulation is similar to that of humans. Pigs possess few innate collateral vessels. However, gradual occlusion of an epicardial coronary artery using an ameroid constrictor induces intramural collateral vessel development that is capable of minimizing myocardial infarction and maintaining normal flow and myocardial function at rest. During severe exercise, however, collateral-dependent flow in the pig is severely compromised such that regional myocardial function is attenuated. The overall response to exercise and functional capacity of coronary collaterals in pigs is similar to the response in humans. We directly assessed collateral-dependent blood flow during a physiological stimulus, ie, near-maximal treadmill exercise. Previously, we have demonstrated that maximal HR in our breed of miniswine is between 260 and 280 beats per minute. We define maximal HR as a plateau in HR despite further increases in external work load or a failure to maintain the external work load. Thus, in the present study, blood flow was measured while our animals exercised at an intensity between 84% and 94% of their maximal HR. This level of exercise was chosen because the animals can maintain a steady state for approximately 4 minutes while the radioactive microspheres are being injected and flushed into the left atrium. Therefore, this exercise intensity represents a physiologically meaningful method of assessing the near-maximal capacity of coronary collateral vessels.

Finally, sonomicrometer dimension gauges were used to assess myocardial function in the collateral-dependent region. This technique allowed us to determine potential functional effects of alterations in collateral-dependent blood flow. Therefore, by using (1) adenosine and adenosine-independent vasodilators, (2) sonomicrometer dimension gauges to document the absence of ischemia during vasodilator administration, (3) a conscious porcine model of collateral development, and (4) techniques for directly assessing collateral-dependent blood flow and regional myocardial function during near maximal exercise, we have overcome the limitations of previous investigations.

Findings from the present study indicate that dipyridamole-treated animals have increased blood flow and decreased ischemic dysfunction in the collateral-dependent myocardium during exercise at 5 and 8 weeks compared with diltiazem-treated and vehicle-treated animals. Since the extent and duration of mechanical stimulation were identical in dipyridamole-treated and diltiazem-treated animals, the possibility exists that adenosine exerted a direct effect to enhance coronary collateral vessel development. There is evidence from in vitro and in vivo studies to support this possibility. For example, adenosine is capable of stimulating endothelial cell proliferation and migration in vitro. In addition, long-term vasodilation using adenosine results in enhanced myocardial capillary density in rabbits. The precise mechanism(s) responsible for the vascular response to adenosine is unclear. However, it has been suggested that adenosine could promote endothelial cell proliferation by potentiating the vascular response to other intrinsic growth factors.

Our secondary hypothesis that improved collateral blood flow is accompanied by histological evidence for capillary angiogenesis was disproved. Previous investigations have indicated that capillary density is increased after chronic administration of dipyridamole in rats and rabbits. These investigators attributed this angiogenic response to mechanical stimulation and/or a direct effect of adenosine. Increased capillary density could augment collateral flow by providing new collaterals originating as sprouts from capillary vessels. Previously, however, we observed that collateral development could occur without increases in transmural capillary density. These results suggested that collateral development occurred from growth and maturation of preformed Anastomotic channels rather than from growth of new vessels. In light of evidence suggesting that adenosine is an angiogenic stimulus, we originally predicted that chronic dipyridamole administration would stimulate new capillary growth. Results from the present study indicate that regional and transmural myocardial capillary density were unaltered in animals receiving dipyridamole. It could be argued that capillary density may have been stimulated in the diltiazem-treated and vehicle-treated animals, thus contributing to the fact that no differences were observed. We feel this to be unlikely, however, since capillary density in these two groups was identical to a control group of animals used in a previous investigation. The discrepancy between our findings and those of others may be attributed to the different animal models used and/or the varied dipyridamole dosing regimens.

We chose capillary density as our index for angiogenesis since it is a well-established and accurate marker and because previous investigations indicate that this variable potentially may increase during long-term dipyridamole and adenosine infusion. Moreover, a recent review suggests that angiogenesis in both heart and skeletal muscle occurs as a result of capillary sprouting from angioblasts. However, although capillary growth provides an acceptable index of angiogenesis, there is evidence to suggest that increased mitotic activity, presumably indicating growth, can occur in other segments of the vasculature (ie, larger vessels) earlier than 8 weeks after placement of an ameroid occluder. We have demonstrated previously that collateral development, as assessed by near-maximal collateral blood flow during exercise, largely occurs...
within 3 to 4 weeks after placement of an atheroid occluder.5,37 Five weeks, therefore, represents the initial period of stabilization after collateral development. Eight weeks represents a period of stable collateral flow, during which we assessed the response of large and small vessels to chronic repeated infusion of a vasodilator. Our data suggest that angiogenesis did not occur. However, growth of larger vessels appeared to occur early (ie, before 5 weeks) during collateral development but not later (ie, 5 to 8 weeks), when collateral blood flow of unstimulated vessels remains stable.5,6,37

A possible explanation for the finding that dipyridamole-treated animals had greater collateral-dependent blood flow during exercise could be that dipyridamole exerted greater mechanical stimulation than did diltiazem. However, we measured transmural myocardial blood flow in the native and collateral circulations after 5 and 8 weeks during infusion of these pharmacological agents. Results confirmed that both vasodilators elicited similar increases in transmural blood flow. LAD blood flow velocity, and mechanical stimulation. Since verification of the acute blood flow responses to dipyridamole, diltiazem, and vehicle were performed in a subset of animals, a potential criticism is that transmural blood flow ratios and LCx systolic wall thickening observed during exercise may be different compared with those for the entire group of experimental animals. However, transmural blood flow ratios and regional myocardial function at rest and during exercise in this subset of animals were similar to those reported for all animals in Fig 3.

An additional concern could be that vasodilation preferentially occurred in the native coronary circulation, causing a coronary steal from the collateralized region, thereby eliciting myocardial ischemia that contributed to collateral development in the dipyridamole-treated animals. We feel this possibility to be unlikely since blood flow increased to the same extent in the control and collateral-dependent regions and since LCx systolic wall thickening was not attenuated throughout administration of either vasodilator. Moreover, collateral-dependent blood flow and myocardial function were not enhanced in the diltiazem-treated animals despite the fact that the extent and duration of vasodilation was similar to the dipyridamole-treated animals.

Dipyridamole infusion is capable of stimulating prostacyclin production in the forearm vasculature of humans.59 In addition, PGE2 elicits angiogenesis in the chick chorioallantoic membrane.60 Therefore, concern could be raised that dipyridamole administration enhanced collateral development through a PG-mediated mechanism. To investigate this possibility, blood samples were obtained from the coronary sinus before and during dipyridamole infusion for the measurement of 6-keto-PGF1α, the stable metabolite of prostacyclin, and PGE2. Since we observed no increase in either PG, we feel that any contribution from these sources to collateral development is unlikely.

Finally, it could be argued that development of collateral-dependent blood flow was inhibited by chronic administration of diltiazem and vehicle rather than stimulated by chronic dipyridamole treatment. This explanation seems unlikely, however, since transmural blood flow ratios obtained during exercise at 8 weeks in the diltiazem-treated animals (0.60±0.07) and vehicle-treated (control) animals (0.62±0.02) were similar to those reported in a control group of animals used previously (0.59±0.06).6

In summary, data presented here do not confirm our original hypothesis that repeated mechanical stimulation alone enhances coronary collateral development in our model. When near-maximal capacity of the collateral vessels was assessed during exercise, we found that blood flow and regional myocardial function were greater in dipyridamole-treated compared with diltiazem-treated and vehicle-treated animals. In addition, we observed no alterations in transmural myocardial capillary density among the three groups of miniswine. Therefore, we believe that increased collateral-dependent blood flow resulted from a direct effect of adenosine and/or from an adenosine-potentiated vascular response to repeated mechanical stress. Moreover, we feel that increased collateral flow in the dipyridamole-treated animals resulted from remodeling of preexisting anastomotic channels rather than from formation of new vessels, since our index of angiogenesis (ie, capillary density) was not altered among the experimental groups.

Acknowledgments

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References

Repeated dipyridamole administration enhances collateral-dependent flow and regional function during exercise. A role for adenosine.
J D Symons, E Firoozmand and J C Longhurst

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