Chronic Reduction of Myocardial Ischemia Does Not Attenuate Coronary Collateral Development in Miniswine

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Background. Myocardial ischemia is considered to be a possible stimulus for development of the coronary collateral circulation. We therefore hypothesized that chronic reduction of myocardial oxygen demand to lessen ischemia would attenuate coronary collateral development over an 8-week period using left circumflex coronary artery (LCx) ameroid-induced constriction in pigs.

Methods and Results. Collateral development was assessed by myocardial blood flow (radioactive microspheres) and left ventricular regional function (sonomicrometer dimension gauges). β-Adrenoceptor blockade with propranolol (160 or 320 mg b.i.d. p.o.) was initiated in 15 animals 1 day after surgery. Compared with 16 untreated animals, β-adrenoceptor antagonism was documented in the treated group by 1) pharmacological stimulation with isoproterenol, 2) physiological stimulation during graded treadmill exercise, and 3) repeated long-term biotelemetry recordings of oxygen demand (heart rate and blood pressure) and regional myocardial function. In addition to pharmacological and physiological verification of β-blockade, biotelemetry showed that, compared with the untreated animals, propranolol significantly reduced the daily number, individual duration, and severity of events representing myocardial dysfunction. This suggests that in the β-blocked group, little if any ischemia was present throughout the first 5 weeks when collateral growth occurs. Transmural myocardial blood flow (expressed as a ratio of flow in the LCx region to the nonoccluded region of the left ventricle) and systolic wall thickening in the LCx region were determined at rest and during treadmill exercise (240 beats per minute) 31–38 days (5 weeks) and 60–67 days (8 weeks) after surgery. Propranolol was withdrawn 3 days before flow and function determinations and was resumed immediately after testing. Blood flow ratios at 5 weeks decreased similarly from rest to exercise in the untreated (0.83±0.04 to 0.60±0.05, p<0.05) and β-blockade group (0.82±0.09 to 0.57±0.10, p<0.05). Systolic wall thickening from rest to exercise was attenuated to the same degree in the untreated (59±6% to 38±6%, p<0.05) and β-blockade group (50±8% to 30±5%, p<0.05). Similar flow and function responses were observed in both groups at 8 weeks.

Conclusions. We conclude that growth and development of the coronary collateral circulation measured functionally during exercise at 90% of maximal heart rate is unrelated to the extent and duration of myocardial ischemia in this model. (Circulation 1992;86:660–671)

Key Words • occlusion, ameroid • wall motion, regional • angiogenesis • blood flow, coronary • growth, capillary

The coronary collateral circulation supplies blood flow to regions of myocardium distal to an arterial occlusion.1,2 The importance of these vessels lies in their ability to prevent myocardial cell death and limit myocardial dysfunction at rest and, to a lesser extent, during exercise.3 Innate collateral vessels are extremely sparse in humans, but enhanced vascular development may occur and become angiographically evident when coronary artery narrowing exceeds 90% of normal.3 Animal models of the coronary collateral circulation have been used to provide a better understand-
illary-like channels. However, enlargement of preexisting vessels seems equally or more likely because collaterals generally are much larger than capillaries and develop over a shorter period than would be required for collaterals to originate from newly formed vessels.

One factor that may be important in collateral development is myocardial ischemia. It is thought that both intensity and duration of ischemia are determinants of collateral growth. Mitogenic factors produced during ischemia, which could act to increase the size of this vascular network, potentially may contribute to enhanced collateral flow. In this regard, ischemia also could serve as a trigger for other stimuli (i.e., adenosine) that may be responsible directly for collateral and capillary development in the heart.

The ability of \( \beta \)-adrenoceptor antagonists to lessen oxygen demand and hence ischemia provides a means to test the requirement for ischemia as a necessary stimulus for vascular development. \( \beta \)-Adrenoceptor blockade reduces myocardial oxygen demand by lowering heart rate, blood pressure, and myocardial contractility. Neill et al. examined the possibility that long-term propranolol administration would reduce myocardial ischemia and collateral growth in a canine model of the coronary collateral circulation. However, although the authors concluded that \( \beta \)-blockade did not affect the development of collateral vessels, this study had several limitations. First, the pacing paradigm used to test the functional capacity of the collateral circulation probably did not stimulate a maximal hyperemia. Therefore, it is uncertain whether the maximal physiological capacity of the collateral circulation was evaluated. In addition, because absolute collateral blood flow was not measured, the extent of the vasodilator response to pacing is uncertain. Second, this investigation used dogs, which are known to develop an extensive collateral circulation consequent to ameroid occlusion. The considerable collateral circulatory response of this species is believed to be in excess of the response in humans. With such extensive collateral vessel development, small reductions in collateral reserve may not be detected because the capacity of the vessels still exerts the demand for flow. Third, myocardial function in the collateral-dependent region was not measured. This would have provided important confirming evidence to substantiate that collateral vessel growth was not altered. Such measurements are necessary to confirm the physiological relevance of the flow measurements. Finally, they did not determine whether their dose of propranolol actually reduced the duration and/or extent of myocardial ischemia.

In light of limitations with the previous study, the purpose of the present investigation was to evaluate the effect of chronic \( \beta \)-adrenoceptor blockade on development of the coronary collateral and capillary circulations in response to gradual occlusion of the left circumflex (LCx) coronary artery. We hypothesized that long-term \( \beta \)-adrenergic suppression with propranolol would reduce the intensity and duration of ischemia and thereby attenuate both collateral vessel development and the resulting ischemically related regional dysfunction in the collateral-dependent myocardium that occurs during exercise. In addition, we predicted that there would be no alteration in capillary density in miniswine after chronic \( \beta \)-adrenoceptor blockade. A preliminary communication of this work has been presented.

### Methods

Surgical and experimental protocols used in this investigation were approved by the Animal Use and Care Committee at the University of California Davis. Thirty-five miniswine were used in the study. During a 2-week period preceding surgery, animals were familiarized with human handling, transport procedures, walking and running on the motorized treadmill, and wearing a protective jacket. Each animal was housed individually in pens and fed twice daily.

### Surgical Procedure

One day before surgery, animals were given an oral dose of Tribrissen (400 mg sulfamethoxazole, 80 mg trimethoprim) and fasted overnight. Immediately before surgery, animals were sedated with ketamine (25 mg/kg i.m.), atropine (0.05 mg/kg i.m.), and sodium thiamylal (20 mg/kg i.v.). They were intubated and maintained on 1–2% isoflurane (Anaquest, Madison, Wis.) anesthesia during an aseptic surgical procedure. Before the first incision, cefazolin sodium (Kezbol 0.5 mg i.m.) was injected into the gluteal region. A left lateral thoracotomy was performed at the fifth intercostal space, and animals were instrumented with left atrial and proximal descending aortic catheters, a high-fidelity left ventricular pressure transducer (Konigsberg P 6.5), and four recording electrodes (one each sewn directly on the epicardial surface of the left atrial appendage, LCx and left anterior descending [LAD] region, and apex of the left ventricle). The proximal LCx was dissected free from surrounding tissue for a distance of 1.0–1.5 cm. Subsequently, a metal-encased ameroid constrictor (K-G Ulrich, Montreal, Quebec, Canada) was placed around the vessel. The size of the constrictor (2.0–3.0-mm lumen) was determined at surgery so that the lumen would provide a close but nonocclusive fit around the artery. Gradual occlusion of the LCx progresses to complete stenosis within 2–3 weeks.

Sonomicrometer dimension gauges (J.W. Inc., San Diego, Calif.) (5 MHz, 2.5 mm in diameter) were placed across the left ventricular free wall for measurement of wall thickness. The subendocardial crystal of the pair was held at the tip of a Teflon tube (1.75 mm o.d.) 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. In 20 animals, two sets of crystals were placed 1–2 cm below the LCx coronary artery, distal to the ameroid constrictor. These were used to assess regional myocardial function in the collateral-dependent region. In 11 animals, a third pair of crystals was placed in the LAD region of the myocardium between the main coronary artery and a diagonal branch. In all animals, crystal placement was confirmed by the presence of decreased wall thickening in the LCx region in response to a brief occlusion (15–20 seconds) of the LCx at the level of the constrictor. Four additional sham control animals were instrumented as above except that after dissection of the LCx, an ameroid constrictor was not placed around the vessel. The sham animals also had a third pair of crystals placed in the LAD region of the myocardium.
Before closing the chest, 1 ml of marcaine hydrochloride (with epinephrine 1:200,000) was injected on either side of the incision to act as a local anesthetic. All catheters and wires were exited from the chest, tunneled under the muscle, and exteriorized on the animal’s back. Three sets of wire sutures were used to approximate the ribs, and the chest was closed in three layers. After closure, cefazolin sodium (Kefzol 0.5 mg i.m.) again was administered.

After extubation and a 2-hour observation period, animals received buprenorphine (0.3 mg i.m.). They then were refitted with custom-made jackets (Alice King Chatham, Los Angeles) to protect catheters and wounds from external perturbation and transported to maximum isolation rooms for 4–6 days to monitor recovery.

**Postsurgical Care**

Animal behavior was monitored at least twice daily, 7 days per week, by laboratory personnel. Tribrisson was administered orally each day throughout the protocol. Catheter exit sites were cleaned and rectal temperatures were obtained three times per week.

**Experimental Protocol**

After surgery, animals were separated randomly into a control group (body weight at surgery, 29.4±1.5 kg; seven male, nine female) and a β-adrenoceptor blockade (propranolol, Inderal LA, Ayerst) group (body weight at surgery, 27.3±2.3 kg; 10 male, five female). Animals in the β-blockade group received either 160 (n=5) or 320 (n=10) mg b.i.d. p.o. 7 days per week for 8 weeks, commencing 1 day after surgery. The propranolol was mixed with the animals' feed, and complete consumption was verified. Four male pigs (body weight at surgery, 26.2±2.5 kg) served as sham controls and were not instrumented with an aneroid constrictor.

Throughout the 8-week protocol, animals were placed on the treadmill at least 1 day per week, and all instrumentation signals were monitored at rest by an eight-channel recorder (Gould TA 4000, Cleveland, Ohio). This monitoring served as a check for all instrumentation signals, documented any changes in hemodynamic function at rest, and maintained familiarization with handling procedures. To maintain familiarization with the treadmill procedure, all pigs ran for 10 minutes 1 day per week, commencing 2 weeks after surgery.

**Documentation of β-Adrenoceptor Blockade**

**Isoproterenol dose–response relation.** Four doses of isoproterenol (0.02, 0.20, 1.0, and 2.0 μg/kg) were administered randomly to control animals and to those receiving propranolol while the animal stood quietly on the treadmill. The chronotropic response was measured from the ECG signal before injection and every 30 seconds for at least 3 minutes after the injection or until no further increase was evident. The 6-second period showing the largest increase in heart rate (HR) was defined as the maximal response.20 HR was allowed to return to baseline (defined as the initial resting HR) between each injection. Dose–response relations were performed 5 and 8 weeks after surgery.

**Hemodynamic response to treadmill running.** Hemodynamic variables were monitored at rest and during treadmill running at 3 mph and 2% grade, 3.2 mph and 5% grade, and 3.4 mph and 8% grade. Each work load was maintained for 3 minutes. Blood pressure, HR, rate–pressure product (RPP), and the first derivative of left ventricular (LV) pressure (dP/dt) at a developed pressure of 40 mm Hg (dP/dt at DP0) were measured at rest and during the last minute of each work load. The dP/dt at DP0 was used because this index of cardiac function is more independent of changes in preload and afterload than maximal dP/dt.21 RPP was obtained by multiplying the systolic blood pressure times HR. Animals in the control group and those receiving 320 mg propranolol b.i.d. performed all three work loads. Animals in the group that received 160 mg b.i.d. propranolol performed only the 3 mph and 2% work load.

**Biotelemetry.** The biotelemetry system consisted of a miniaturized, dual-channel, ultrasonic transit-time dimension circuit in conjunction with a blood pressure amplifier.22 This system provided continuous measurements of LV blood pressure and systolic wall thickening (Wth) while the animals were unrestrained in their pens. LV blood pressure was obtained from the LV Konigsberg transducer and systolic Wth from ultrasonic dimension gauges placed in the LCx and LAD regions. The animals used for biotelemetry carried the device in a pouch attached to their protective jacket. The telemetered data were recorded continuously for approximately 18-hour periods on an analog instrumentation tape recorder (Hewlett-Packard 3968 A). Data on analog tape were digitized at a rate of one sample every 625 μsec, using a data acquisition and analysis system (EGAA, RC Electronics, Coleta, Calif.) and microcomputer (ALR 25 MHz 80386). Digitized data were then stored on a 650-Mbyte erasable laser disk (RO-503E, Relax Technology, Union City, Calif.).

Initial data analysis used a data reduction function built into the EGAA system. The data reduction function accepted inputs from the two systolic Wth and the blood pressure measurements and calculated HR, systolic left ventricular blood pressure (SBP), dP/dt, and percent systolic Wth.

The extent and duration of myocardial dysfunction was measured using a software program designed in our laboratory (Figure 1).23 Average control values of LCx Wth, LCx Wth/LAD Wth, and LCx Wth/RPP were determined for each individual recording period. These values comprised an initial baseline. By iterating through a decision-making tree, a binary classification algorithm classified each individual data point as being above or below each respective initial baseline. An adjusted baseline for each of the three variables then was calculated using the mean of all data points above the initial baseline. This adjusted baseline, referred to as the control value, was thought to most accurately represent the mean value for each variable because it excluded the periods of myocardial dysfunction. Dysfunction in the LCx region occurred when three criteria were simultaneously satisfied for at least 30 seconds. These criteria included a decrease from control values in LCx Wth, the LCx Wth/LAD Wth ratio, and the LCx Wth/RPP ratio. Satisfaction of all three criteria was termed an event representing myocardial dysfunction. The total duration of dysfunction during each recording was determined by summing the time period of each event and dividing by the total daily recorded time. Therefore, duration of myocardial dysfunction is ex-
pressed as a percentage of total recorded time. The extent of dysfunction was represented by the mean decrease from control values in LCx Wth during each event and is expressed relative to the control LCx Wth, which was arbitrarily determined to be 100%. The average length of each recording period was 17.7±0.5 hours for the untreated group, 18.6±0.4 hours for the treated group, and 19.1±0.4 hours for the sham animals, respectively. The average number of cardiac cycles recorded each day was 59,548±3,387 for the untreated group, 56,978±1,998 for the treated group, and 55,190±3,335 for the sham animals. Recordings began at approximately 8:00 AM.

**Regional Blood Flow**

Myocardial blood flows were determined during weeks 5 (31–38 days after surgery) and 8 (60–67 days after surgery) while the animal stood quietly on the treadmill and at a treadmill speed and grade sufficient to increase the animal’s heart rate to approximately 240 beats per minute. This exercise intensity was chosen because it elicits near-maximal physiological capacity of the collateral vessels and can be maintained as a steady state for approximately 4 minutes during determination of blood flow. Propranolol was withdrawn from the treated group 3 days before determination of blood flow and was resumed immediately after the test. Aortic pressure, LV pressure, dP/dt, ECG, and somnicrometer dimension signals were monitored during flow determinations. Hemodynamic variables measured immediately before and after each particular microsphere injection were similar and therefore were combined. In addition, because the hemodynamic values were similar for animals that had received both doses of propranolol, results were pooled and the combined groups are referred to as the β-blockade group. Arterial blood lactate (model 23L, Yellow Springs Instruments, Yellow Springs, Ohio) and blood gases (ABL3 Radiometer, Copenhagen) also were obtained. Regional myocardial blood flow was measured by injecting approximately 3x10^6 15-μm radiolabeled microspheres (New England Nuclear, Boston) into the left atrium. Spheres were suspended in 10% Dextran and 0.01% Tween 80 solvent and agitated with a vortex mixer before injection. The radio-labels that were used included scandium-46, niobium-95, indium-114, ruthenium-103, tin-113, chromium-51, cerium-141, or strontium-85. Reference blood was withdrawn (Sage, Model 351, Cambridge, Mass.) from the aortic catheter at 8 ml/min for 150 seconds. Hemodynamic values were monitored continuously to document a steady state during blood flow determinations. Regional myocardial and renal blood flows were calculated according to the method of Heymann et al.25 Myocardial and reference blood samples were analyzed for the quantity from each isotope of gamma radiation using a germanium detector (Micrad Inc., Knoxville, Tenn.). Blood flows were normalized per gram of myocardium.

Similar blood flows were measured in epicardial (epi), midmyocardial (mid), and endocardial (endo) layers, comparing the LAD and right coronary artery (RCA) regions. Therefore, these two regions were combined and are referred to as the control region. The area perfused by the LCx is referred to as the collateral region. The transmural flow ratio was obtained by dividing transmural flow in the collateral region by transmural flow in the control region. The endo/epi flow ratio also was calculated.

Blood flows in the cortex and medulla of each kidney also were determined to document adequate mixing of the microspheres. Percent differences in blood flow between the right and left kidney were <15%, documenting adequate mixing.

**Regional Myocardial Function**

Regional myocardial function was assessed with somnicrometer dimension gauges. Wth was measured over the ejection phase of the systolic cycle from the onset of ventricular ejection to the end 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 (n=6, both groups), the ejection phase was defined as the time from peak dP/dt to 20 msec before peak negative dP/dt.26 In animals without 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 previously27 that no difference exists between dP/dt or the ST interval when calculating the systolic time interval. Data are expressed as Wth% calculated as end-systolic Wth (ES Wth) minus end-diastolic Wth (ED Wth) divided by ED Wth times 100. All hemodynamic and regional function calculations were determined over five and 10 consecutive cardiac cycles during rest and exercise, respectively. Regional myocardial function was measured immediately before and after each particular microsphere injection. Because no differences existed between these values recorded during both periods, data were combined.

**Euthanization Procedures**

To document complete closure of the am eroid constrictor, an angiogram of the left coronary artery system was performed (Siremobil 4U, Siemens, Iselin, N.J.; Fluoro 100 Series Digital Image Processor, Eigen, Ne-
vada City, Calif.). Data from one animal in the control group and one animal in the \( \beta \)-adrenoceptor blockade group were not included in the analysis because of incomplete closure of the amiodar constrictor.

The heart was arrested in diastole using saturated KCl. After rapidly excising the heart from the chest, the aortic root was perfused retrogradely with 1 l of phosphate buffer and 2 l of phosphate-buffered glutaraldehyde. Catheters then were placed in the proximal LAD and RCA. The LCx was cannulated just distal to the amiodar occluder. Phosphate-buffered glutaraldehyde (\( \approx 50 \) ml) then was perfused through each coronary artery. Two different colored dyes (Sigma Chemical, St. Louis, Mo.) were added to the glutaraldehyde (\( \approx 150 \) ml) and injected into two of the catheters while clear glutaraldehyde was injected into the third to delineate the regions of myocardium subserved by the LAD, LCx, and RCA. Finally, the heart was suspended in buffered glutaraldehyde for at least 24 hours before sectioning.

Before sectioning, the fat and major vessels were trimmed from the myocardium, which then was cut into five transverse rings (1-1.5 cm thick) along the hoop axis from base to apex. Tissue from the two most basal rings (1 and 2) were used in the analysis of myocardial blood flow. Using the dyes and sonomicrometer dimension gauges as markers, each ring was divided into the LAD, RCA, and LCx perfusion territory. The septum was excluded from both control regions. Each individual perfusion bed then was divided such that there were two border zones (defined as containing two perfusion territories) and a central zone. To ensure that tissue was from areas within the center of each perfusion bed and to reduce the potential problem of inaccurate flow resulting from fingers of tissue from one bed extending into another, only blood flow from the central zone was used in the statistical analysis. The central zone of each perfusion bed was divided transmurally into three pieces of approximately equal thickness corresponding to the endo, mid, and epi layers. Average weights of each piece of tissue from the collateralized region were 1.26±0.13 g, 1.16±0.13 g, and 1.70±0.26 g for the endo, mid, and epi 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.\(^{28}\) Epi, mid, and endo tissue sections were embedded in paraffin, cut (4 \( \mu \)m) using a microtome, and stained with Masson’s trichrome stain to identify fibrous necrotic tissue.\(^{29}\) Each slide was point-counted, and the percent of necrotic tissue was measured. A sheet containing a series of 95×95-\( \mu \)m grids was projected onto the field of a microscope (Leitz Wetzlar) overlaying 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

\[
\frac{V_v}{V_{tissue}} = \frac{P_v}{P_{tissue}}
\]

where \( V_v \) is the volume of necrotic tissue, \( V_{tissue} \) is the volume of the total tissue sample, \( P_v \) is the number of points lying over necrotic tissue, and \( P_{tissue} \) is the total number of points lying over the total tissue sample.\(^{30}\)

Capillary density (vessels per square millimeter) was measured after hematoxylin/eosin-stained, 2-\( \mu \)m plastic-embedded sections were cut perpendicular to the fiber orientation using a JB-4 microtome (Dupont, Sorval).\(^{31}\) Perfusion-fixation facilitated the counting of capillaries because they were fixed in an open position. Capillary density was determined by counting the number of capillaries located within a series of test grids (95\( \mu \)m×95\( \mu \)m). The analysis comprised at least 20 fields in two blocks of tissue from both rings 1 and 2 for percent infarction and capillary density.

**Statistical Analysis**

Data are expressed as group mean±SEM. A two-way ANOVA with repeated measures over time was used to examine the responses to isoproterenol, treadmill running, and biotelemetry. Post hoc tests (with Bonferroni correction for multiple tests) were performed when significant interactions between group and time were obtained (\( p<0.05 \)).\(^{31}\) Comparison of hemodynamic variables, transmural flow ratios, regional blood flows, regional myocardial function, blood lactate, capillary density, and infarction were made using a two-way ANOVA. When significant main effects were obtained, post hoc analyses were performed as described above.

**Results**

The percent tissue infarction for control animals averaged 5.5±0.8%, 6.1±1.1%, and 6.1±0.9%; for the \( \beta \)-blockade group it averaged 7.6±1.4%, 7.9±0.8%, and 7.6±0.9% in the epi, mid, and endo layers, respectively. The extent of infarction in any layer was not significantly different between the two groups.

**Documentation of \( \beta \)-Adrenoceptor Blockade**

Figure 2 shows the chronotropic response to isoproterenol. For each dose injected, the maximal HR change was significantly higher in the control animals compared with the \( \beta \)-blockade groups. Animals treated with the highest dose of propranolol, 320 mg b.i.d., demonstrated smaller chronotropic responses at the highest doses of isoproterenol compared with the 160-mg group. Figure 3 illustrates the hemodynamic responses at rest and during exercise. Before exercise, HR and \( dP/dt \) at \( DP_{min} \) were lower in the 320-mg group compared
with the untreated group. RPP was similar among the three groups. During exercise, HR, RPP, and dP/dt at DP at 8 weeks for the control group and 8-blockade group, respectively. Because treadmill speed and grade, time to reach 240 beats per minute, blood lactate, and both rest and exercise hemodynamics were similar between the groups, no residual effects of propranolol were present in the 8-blockade group during the 5- and 8-week blood flow determinations.

Regional Myocardial Blood Flow

Absolute blood flows and endo/epi blood flow ratios for animals in the control and 8-blockade group are presented in Table 2. Blood flows in all regions for both groups increased significantly during exercise. Flow during exercise, however, was significantly less in the collateral region compared with the control region. We observed lower flows in the control but not the collateral region of the 8-blockade compared with the untreated animals in mid and endo regions only at 5 weeks. However, a similar difference was observed in the epi layer of the collateral region at 5 weeks. The endo/epi blood flow ratios were significantly lower during exercise than at rest in the control region of both groups and in the collateral region of the untreated group at 5 weeks. At 8 weeks, the endo/epi flow ratio decreased during exercise in the collateralized region of the untreated group. In the 8-blockade group, blood flow in
the epi and mid layers of the control and collateral regions was greater during exercise at 8 compared with 5 weeks.

Transmural flow ratios, which normalize for interanimal variability, obtained at 5 (panel A) and 8 (panel B) weeks are presented in Figure 5. Resting flow ratios and the decrease in flow ratios during exercise were similar for both groups.

**Regional Myocardial Function**

Percent Wth at rest and during exercise for the control and β-blockade groups are presented in Figure 6. End-systolic and end-diastolic Wth at rest for the control group were 12.7±1.2 and 8.0±0.6 mm, respectively, at 5 weeks and 12.8±1.3 and 8.3±0.8 mm at 8 weeks, and for the β-blockade group were 14.8±0.8 and 10.0±0.7 mm at 5 weeks and 15.5±0.7 and 9.8±0.6 mm at 8 weeks. During exercise, there was a significant decrease in Wth in the LCx region. In the control animals, end-systolic and end-diastolic Wth were 12.1±1.3 and 8.5±0.6 mm, respectively, during exercise at 5 weeks and 12.0±1.3 and 8.8±0.7 mm at 8 weeks; in the β-blockade group they were 13.7±0.8 and 10.5±0.6 mm at 5 weeks and 14.4±0.7 and 10.7±0.4 mm at 8 weeks. Although there was a significant decrease in LCx regional Wth during exercise, the magnitude of this decrease was similar in the control and β-blockade groups. Data obtained from animals in which sonomicrometer dimension gauges were placed in the LAD region indicated no difference in Wth when rest (44.9±8.3%) was compared with exercise (43.4±9.7%).

**Capillary Density**

There were no differences in capillary density between the control and β-blockade group (Table 3).

**Discussion**

Results from this study do not support our original hypothesis that chronic β-adrenoceptor blockade with propranolol attenuates the growth and development of coronary collateral vessels in miniswine. Because propranolol virtually eliminated all myocardial dysfunction, and because dysfunction in untreated animals probably was caused by repeated episodes of ischemia, these data strongly suggest that ischemia is not required for development of these vessels. Despite the normal growth and development of coronary collaterals, we observed that transmural capillary density was unaltered by β-adrenoceptor blockade. Thus, we found no evidence for angiogenesis. Collateral development in LCx-occluded mini-
A treated group therefore does not depend on the presence of repeated ischemia and probably results from enlargement of preformed anastomotic connections rather than an angiogenic process.

To adequately explore our hypothesis that ischemia is both an important and a required stimulus for coronary collateral vessel growth, it was necessary to show that we could substantially reduce, or virtually eliminate, both the extent and the duration of ischemia. We initially proposed to accomplish this task by testing the efficacy of β-adrenoceptor blockade pharmacologically and physiologically. Injection of isoproterenol and graded exercise testing provided evidence that animals were significantly β-blocked and that the extent of receptor antagonism was related to the dose of propranolol. However, these types of tests confirm blockade but provide little information about the effect of propranolol on the frequent episodes of myocardial ischemia occurring throughout the day and possibly at night. For instance, we have noticed that large hemodynamic responses occur in pigs when they are being handled, transported, or fed. These hemodynamic responses can be severe enough to cause arrhythmias, presumably related to ischemia and consequent sudden death. One might assume logically that propranolol, through its β-adrenoceptor antagonism, resulting in reduced heart rate, blood pressure, and contractility, would lessen myocardial oxygen demand and hence reduce ischemia throughout a 24-hour period. We believed this assumption to be correct because we used a long-acting preparation of propranolol that was designed to effect a continuous reduction in myocardial oxygen demand and ischemia.

To more thoroughly examine the question of whether our dose of propranolol reduced myocardial oxygen demand and myocardial ischemia, we developed a method of continuously monitoring the RPP as well as regional myocardial function in the intact, instrumented swine. The RPP provides an indication of myocardial oxygen demand, whereas regional myocardial function (or dysfunction) using ultrasonic dimension gauges affords an index of the balance or imbalance between oxygen supply and demand, i.e., myocardial ischemia.

To continuously determine the extent and duration of myocardial dysfunction, we used an iterative decision-making tree to classify each data point, i.e., each cardiac cycle, as either being normal or exhibiting dysfunction. To accomplish this, an initial baseline of resting function was determined by using the average value obtained throughout the entire recording period. An adjusted baseline was obtained by using the average of all values above this initial baseline. Myocardial dysfunction was recorded for every series of cardiac cycles greater than 30 seconds in duration, which decreased below this adjusted baseline, occurred in the collateral-dependent (LCx region) but not the freely perfused (LAD) region (i.e., a decrease in LCx Wth/LAD Wth), and occurred at a time when the RPP was either stable or increased (i.e., a decrease in LCx/RPP). The latter two criteria helped to eliminate false classification of myocardial dysfunction. For example, we were able to distinguish between a reduction in function consequent to a decrease in oxygen demand as opposed to relative dysfunction in the LCx compared with the LAD region, which occurs when oxygen demand remains constant or is increased. To test the specificity of our algorithm, four sham animals were studied over the same time period as the two ameroid-constricted groups. We observed that dysfunction in the sham animals, as defined by our algorithm, occurred less than 1% of the time over the 5-week period. Conversely, in the untreated animals, dysfunction occurred approximately 20% of the time. Therefore, we believe our algorithm to be quite specific for dysfunction, which is probably related to episodes of myocardial ischemia associated with gradual and complete occlusion of the coronary artery by the ameroid constrictor.

An important assumption in our study is that myocardial function closely reflects myocardial ischemia. As...
transmural blood flow decreases, systolic Wth is attenuated, and myocardial lactate production occurs.32 Lactate production is considered to be a good indicator of myocardial ischemia. One study, using isotopically labeled lactate, indicated production when myocardial function was reduced by 20% but not when function was reduced by 5%.32 Our results from 5 weeks of biotelemetry data indicate that untreated ameroid-occluded animals averaged 20% dysfunction (i.e., 80% of normalized function) for approximately 20% of each 18-hour recording period. Both the extent and duration of dysfunction were significantly higher compared with the sham and treated animals. Animals in the β-adrenerceptor blockade group averaged 6% dysfunction (94% of normal function) for only 5% of each recording period. Although the duration of dysfunction in the propranolol-treated animals was higher during three of the 10 recording intervals compared with the sham group (Figure 4, panels A and B), the extent of ischemia was similar to sham animals. A previous report suggests that the extent of insignificant dysfunction (=6%) that remained in the treated group is incapable of eliciting metabolic ischemia.32 Thus, our index of metabolic ischemia, regional myocardial function, indicates that animals treated with propranolol experienced no physiologically meaningful ischemia.

Both chemical and mechanical factors are considered to be important stimuli in promoting coronary collateral vessel growth. Chemical or mitogenic stimuli are thought to be released in response to ischemia,4,13,34 and their effect is related to the mass of ischemic myocardium.13 Furthermore, the intensity and duration of ischemia also have been considered important factors in determining collateral vessel development.10 Exercise training, for instance, recently has been shown to enhance coronary collateral vessel formation and attenuate regional dysfunction during exercise.7 In this study, it was speculated that the repeated exercise bouts and concomitant ischemia in the collateral-dependent region may stimulate various mitogenic factors concerned with collateral vessel growth. In most previous studies that used gradual or repeated coronary occlusions,6,7,10,13,15,33 ischemia was not the only stimulus that could stimulate collateral vessel development. For instance, a variety of mechanical factors related to flow velocity and intravascular pressure gradients are present and potentially could be responsible for the growth of these vessels.11,16,34,35 Our present data also indicate that there is not a good relation between the extent of ischemia and enlargement of collaterals, at least in the ameroid-constricted miniswine model. Thus, we believe that factors other than chemical stimuli produced during ischemia can play an important role in coronary collat-

eral growth. These may include mechanical forces such as the pressure gradient within these vessels,4,7,13,15,33 flow velocity, or wall stress.7,10

It could be argued that the brief period of time that the animals were withdrawn from propranolol and/or the small amount of insignificant dysfunction that remained in the propranolol-treated animals was sufficient to cause coronary collaterals to develop. Also, it might be suggested that the ischemic episodes in the treated group were missed because we recorded myocardial function only for approximately 18 hours twice each week rather than continuously for 24 hours throughout the first 5 weeks. Biotelemetry data were obtained during the day starting at approximately 8:00 AM to ensure that the majority of potentially ischemic episodes were recorded. These times were considered the most likely for ischemia to occur, as they included periods when animals were being fed, handled, and transported. We also believe it is unlikely that significant ischemic episodes were missed during our twice-per-week recording schedule. The treated animals demonstrated a very constant reduction in ischemia despite the fact that hemodynamic recordings were taken at different times each week throughout the 5-week recording period. Furthermore, the untreated animals demonstrated constant repeated periods of ischemia throughout the initial 5 weeks; that is, we did not observe significant variability in either group with respect to the daily extent or duration of ischemia. Our experimental design also involved administration of propranolol within 1 day of surgery so that we could achieve a significant blood level and physiological response within 24–36 hours of surgery. We have shown previously that this period of time is well in advance of any significant coronary occlusion caused by the ameroid constrictor, even in the small subset of animals that demonstrate rapid occlusion such that they infarct.36 Furthermore, treated animals were maintained on propranolol twice daily for the entire 5 weeks, at which time they were withdrawn for the first series of blood flow experiments. The period of time between stopping propranolol and exercise testing was kept at a minimum (3 days) to ensure that the animals were "ischemic" for a minimal period of time while not demonstrating residual hemodynamic effects of β-blockade at rest or during exercise. In a previous study using dogs, repeated occlusions for an average of 6 days were required to develop coronary collateral vessels.10 Therefore, any ischemic events occurring during the time our treated animals were withdrawn from propranolol probably did not provide sufficient stimulus for vascular growth. Perhaps the most difficult question to answer is whether the small (and statistically insig-

### Table 3. Capillary Density

<table>
<thead>
<tr>
<th></th>
<th>LCx</th>
<th>LAD</th>
<th>RCA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Epi</td>
<td>Mid</td>
<td>Endo</td>
</tr>
<tr>
<td>Untreated (n=10)</td>
<td>2,119±120</td>
<td>2,655±244</td>
<td>2,548±228</td>
</tr>
<tr>
<td>β-Blocked (n=11)</td>
<td>2,672±253</td>
<td>2,816±260</td>
<td>2,946±505</td>
</tr>
</tbody>
</table>

Values are mean±SEM; units are vessels/mm². LCx, left circumflex coronary artery; LAD, left anterior descending coronary artery; RCA, right coronary artery; Epi, epicardium; Mid, midmyocardium; Endo, endocardium.
significant compared with sham animals) amount of residual ischemia in the propranolol-treated group constituted a sufficient stimulus to cause collateral vessel growth. However, posing this question assumes that the reduction in function from an average of 96% of normalized function in the sham animals to 94% in the β-blocked animals represents ischemia. Although the extent of dysfunction was not significantly greater during any of the recording intervals in the β-blocked versus the sham animals, the duration was greater during three of the 10 recording intervals. As we have argued above, based on metabolic studies of lactate production, we do not believe that the greater duration of insignificant ischemia experienced by the propranolol-treated animals represents physiologically meaningful ischemia. However, we cannot entirely discount the possibility that extremely mild albeit insignificant ischemia is capable of inducing collateral vessel formation. Perhaps a mitogen that stimulates collaterals to grow is released before blood flow and regional function are decreased to the point that lactic acid is produced. If this reasoning is true, then one must believe that the ischemic threshold for collateral growth is very low. However, most investigators believe that collaterals are stimulated to grow only by severe ischemia. For instance, collateral growth in humans and other species generally is only found with severe (i.e., total or subtotal) occlusions of major epicardial conductance arteries.3,16 Thus, although future studies need to focus on identifying an ischemic threshold for collateral growth, we believe that the small amount of ischemia in our treated group was unlikely to stimulate vascular development. Therefore, we suggest that although ischemia may be a stimulus for collateral vessel development in the ameroid-constricted pig model, it is not a requirement.

Another possible limitation of our study is that we may not have measured the maximal functional capacity of the coronary collateral vessels. In the present study, animals performed a work load sufficient to raise HR to 240 beats per minute. Previously, we have demonstrated maximal HR in our breed of miniswine to be between 260 and 280 beats per minute.42 Thus, blood flow was measured while our animals exercised at an intensity between 86% and 92% 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. It is possible that a small effect of propranolol to reduce maximal collateral flow capacity would be revealed only during periods of stress that induce maximal collateral vasodilation. In this regard, a previous study in miniswine demonstrated an insignificant increase in subendocardial collateral blood flow when treadmill work load increased HR from 230 to 280 beats per minute during exercise.4 Thus, although we cannot eliminate the possibility that a further increase in collateral flow may have occurred at higher work loads with higher HR, we question whether such a small effect would be physiologically meaningful since we observed substantial dysfunction during exercise in our treated animals. Thus, it is uncertain that a slightly higher work load and associated increase in myocardial oxygen demand would produce more ischemic dysfunction in the collateral region and allow us to detect a subtle effect of β-blockade to reduce maximal functional collateral flow capacity.

There may be additional concern that the miniswine is not a representative model for adult humans for identifying and studying factors concerned with the promotion of collateral vessel growth. For many years, dogs have been studied because they form an abundance of collateral vessels during amiodarone constriction of their coronary arteries.2,10,12,13,18,35 Recently, pigs have been used as a relevant model of the coronary collateral circulation.4-7,35 Miniswine have a coronary circulation that is both anatomically and physiologically similar to humans.16,34,35 Like humans, pigs possess few innate collateral vessels.4,5,34,35 However, after gradual occlusion of an epicardial coronary artery with an ameroid constrictor, swine form intramural collateral vessels that have the capacity to maintain normal flow and myocardial function at rest and to minimize myocardial infarction.4,7 During stress such as exercise, however, collateral-dependent flow in the pig is severely compromised such that regional myocardial function cannot be maintained.4,7 This functional capacity of coronary collaterals in pigs is analogous to that possessed by humans.16,35 Another concern regarding our model is that regional blood flow measurements were obtained in miniswine approximately 6 months old, and results may not be relevant to the adult human. Miniswine are sexually mature at 6 months, but they continue to gain weight for at least 2 years,43 making them chronologically analogous to young adults. Therefore, we consider the pig to be a very relevant model for the study of collateral growth and development; however, caution must be used when extrapolating our results to middle-aged and older adult humans.

In addition to our observation that the extent of ischemic dysfunction was not related to collateral growth and development, we found that there was no effect on transmural capillary density. We are uncertain whether similar mechanisms are operative in growth and development of collateral vessels as with microvessels. These microvessels are thought to grow and proliferate in response to chemical and mechanical stimulation.44-46 This proliferative response, termed angiogenesis, is related to ischemia in certain organs such as skeletal muscle and the retina.47,48 It has been suggested that collateral vessels may originate from sprouting capillaries4 or capillary-like channels6; therefore, we measured the density of these microvessels to provide evidence for angiogenesis. However, by this method we were unable to demonstrate angiogenesis in our model despite the fact that collateral flow development occurred. Our findings contrast with those from a previous investigation, which reported that propranolol treatment increased capillary density in the rabbit myocardium.49 However, species differences and the absence of verification of β-blockade and coronary artery occlusion in the earlier investigation make comparisons with our study difficult.

Our results demonstrate that collateral development is possible in the absence of capillary angiogenesis in the bed at risk. This finding provides evidence that preformed collateral vessels may initially exist and subsequently enlarge in response to gradual occlusion of a coronary artery. These results agree with previous investigations showing enlargement of human30,51 and
animal native collateral vessels in hearts with coronary obstructions.

Summary

Data presented here indicate that virtual elimination of ischemia, as defined by myocardial dysfunction, does not alter the growth and development of coronary collateral vessels. Although we cannot entirely discount stimulation of collateral vessel growth by a mild degree of residual ischemia, we believe that this possibility is unlikely. Therefore, we conclude that ischemia is not essential for collateral vessel growth and suggest that other factors, perhaps mechanical in nature, can stimulate the enlargement of preformed coronary collateral vessels in response to gradual coronary occlusion by an amiodar constrictor.

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