Increase in Collateral Blood Flow Following Repeated Coronary Artery Occlusion and Nitroglycerin Administration

Susan Brazzamano, Arthur E. Mays, Judith C. Rembert, and Joseph C. Greenfield, Jr.

From the Department of Medicine, Division of Cardiology, Duke University Medical Center, Durham, North Carolina, and the Medical Service, Cardiology Section, Veterans Administration Medical Center, Durham, North Carolina

SUMMARY. The effects of occlusion, reperfusion, reocclusion (n = 13), and nitroglycerin (n = 10) on regional transmural myocardial collateral blood flow was tested in conscious dogs in which collateral development was stimulated by partial stenosis of the left circumflex coronary artery. Hemodynamics and collateral blood flow were measured during the awake state using 9-μm radioactive microspheres. Regional transmural flow was measured during transient occlusion of the circumflex artery at 7 and at 14 days postoperatively. On the 14th postoperative day, two sets of circumflex occlusions and blood flow measurements were carried out. The first set consisted of two occlusions separated by 15 minutes. The second set performed 2 hours later included two occlusions, separated by 15 minutes, and nitroglycerin administration. Mean collateral blood flow increased significantly (P = 0.002) from 0.10 ± 0.07 ml/min per g on day 7 to 0.25 ± 0.18 ml/min per g on day 14. A significant increase in mean collateral blood flow from occlusion one to two was observed (0.28 ± 0.17 to 0.37 ± 0.22 ml/min per g, P = 0.005). Mean collateral flow increased significantly (P = 0.01) between pre- to post-nitroglycerin occlusions, 0.28 ± 0.20 to 0.46 ± 0.32 ml/min per g. Although this increase appeared to be greater than during the first set of occlusions, it did not reach statistical significance (P = 0.08). These data indicate that when immature collaterals are present, occlusions, reperfusion, and reocclusions of a major coronary artery produce augmentation in collateral flow. This must be considered in evaluating interventions which may alter collateral flow. (Circ Res 54: 204-207, 1984)

IN the event of coronary artery occlusion, collateral vessels can provide an alternate source of blood flow. Little is known, however, about the functional physiology of immature collateral vessels. If a partially stenosed major coronary artery is suddenly totally obstructed, the integrity of the myocardium will become entirely dependent upon the functional adequacy of the collateral vessels. It is important, therefore, to determine whether immature collateral vessels function to deliver a fixed maximal amount of flow immediately after total occlusion of the stenosed coronary, or if blood flow to the collateral-dependent area can be augmented by prior brief episodes of total occlusion, or by drug administration.

In order to carry out these studies, a model was developed in our laboratory in which collateral vessel growth was stimulated by partial stenosis of the left circumflex coronary artery. In our model, the blood flow to a region of myocardium was supplied by both the partially stenosed circumflex artery, and by immature but developing collateral vessels. During complete temporary occlusion of the circumflex artery, the immature collaterals were the primary source of flow to this area of myocardium.

The primary aim of the study was to determine whether (after a brief occlusion of a major coronary artery and reperfusion), a second occlusion would elicit equal, increased, or decreased collateral flow in addition, to provide a basis for evaluating the results of studies in which multiple coronary occlusions are performed to compare the effects of drugs on collateral perfusion. A second aim was to determine whether nitroglycerin could augment flow to an area of myocardium supplied by immature collateral vessels. These data will have important implications in a complete understanding of the physiology of the collateral circulation.

Methods

Fourteen adult mongrel dogs weighing 16–27 kg were anesthetized with intravenous sodium thiamylal (30 to 40 mg/kg), and underwent a left thoracotomy, using sterile technique. Since the integrity of the study was dependent upon complete occlusion of the circumflex, the proximal left circumflex coronary artery was dissected free and an electromagnetic flow probe (Howell Instruments Inc.) positioned around the vessel in order to verify zero flow during occlusion. A pneumatic occluder constructed in our laboratory was placed distal to the flow probe. The occluder diameter was adjusted as snug as possible without altering the reactive hyperemic response to a 10-second coronary occlusion. Polyvinyl chloride heparin-filled catheters, 3 mm in diameter, were placed in the ascending aorta via the left internal mammary artery, the left atrial
appendage, and the left ventricle, through a stab wound in the apex. The catheters, occluder, and flow probe leads were tunneled through the chest wall, placed in a subcutaneous pouch at the base of the neck, and the thoracotomy was closed. Routine postoperative care, including antibiotics, was followed. The animals were placed in the vivarium to recover.

The animals were brought to the laboratory 7 days postoperatively. Morphine sulfate (10–20 mg) was administered intramuscularly. By sterile technique, the subcutaneous pouch was infiltrated with 2% lidocaine hydrochloride, an incision made, and the instrumentation exteriorized. The aortic, left ventricular, and left atrial pressure catheters were connected to Statham P23Db transducers. The flow probe was connected to a Howell model HMS 1000 flowmeter (Howell Instruments). After 1 hour or more, phasic aortic, left ventricular, and left atrial pressures were monitored continuously and recorded, as were phasic left circumflex coronary artery flow and lead II of the electrocardiogram, on a Hewlett-Packard model 7700 eight-channel direct-wiring oscillograph. The pneumatic occluder was inflated to produce complete occlusion of the left circumflex artery, and continuously verified by the flowmeter. Two minutes after the onset of occlusion, a regional transmural myocardial blood flow measurement was carried out with radioactive microspheres. The occlusion was maintained until the reference sample collection was completed, then was released. The instruments were disconnected and replaced into the subcutaneous pouch.

The animals again were brought to the laboratory on the 14th postoperative day for study. The subcutaneous pouch was reopened and the instrumentation was connected as described previously. After a control basal state was attained, hemodynamic data and regional transmural myocardial blood flow measurements were obtained. Then the coronary artery was occluded for approximately 4 minutes (the microspheres injected at 2 minutes). Fifteen minutes later, the occlusion was repeated, adhering to the identical protocol. A period of 2 hours elapsed. Again, the coronary artery was occluded for approximately 4 minutes in duration, and the same parameters were measured. Ten minutes later, 0.4 mg nitroglycerin (Parke-Davis Corp.), freshly dissolved in 5 ml of normal saline, was administered intravenously over a 30-second period and flushed over 30 seconds with 10 ml of normal saline. Approximately 5 minutes following this nitroglycerin administration, after hemodynamics had returned to baseline, again the coronary artery was occluded for approximately 4 minutes, following the protocol exactly.

Regional transmural myocardial blood flow was measured with 9 ± 1-μm microspheres (Minnesota Mining and Manufacturing Co.) labeled with six different γ-emitting radionuclides, as has been described previously (Swain et al., 1979).

After completion of all data collection, the animals were anesthetized with sodium thiamylal and killed with potassium chloride. Each of the 14 hearts was placed in 10% buffered formalin, fixed, trimmed, and sectioned into six anatomic regions, as described previously (Fedor et al., 1980). Each region was subdivided into four transmural layers from epicardium (layer 1) to endocardium (layer 4). Gross inspection and histological examination of appropriate samples revealed no evidence of myocardial infarction. The individual tissue samples weighing from 0.5 to 1.5 g were placed into separate formalin-filled vials for subsequent counting.

The anatomic region receiving the least blood flow during circumflex coronary artery occlusion on postoperative day 7 was designated the ischemic or collateral-dependent region, assuming this area most dependent upon the developing collaterals for blood flow. This was most often the posterior papillary region. The normally perfused or nonischemic regional flow was obtained as averages from the anterior and anterior papillary regions.

Hemodynamic data were measured directly from the oscillograph recording obtained at the time of microsphere injection. Data in the Results section are expressed as mean ± 1 so. Statistics were based on Student’s t-test for paired data, analysis of variance, and linear regression.

Results

The 13 dogs studied on postoperative day 7, and, again, on postoperative day 14, showed an increase in blood flow to the collateral-dependent region during circumflex occlusion between the two measurements. These dogs were considered to have collateral development stimulated by the partial stenosis of the left circumflex coronary artery. All myocardial blood flow changes are greater than the error associated with the microsphere method for this laboratory (Swain et al., 1979). Mean flow to the collateral-dependent region increased from 0.10 ± 0.07 ml/min per g on day 7 to 0.25 ± 0.18 ml/min per g on day 14 (P = 0.01). Mean flow to the normally perfused myocardium was 1.04 ± 0.42 ml/min per g on day 7, 1.4 ± 0.64 ml/min per g on day 14, and was not different (P = 0.59). Hemodynamic parameters during occlusion were not statistically different on postoperative days 7 and 14.

The effect of occlusion, reperfusion, and reocclusion on postoperative day 14 in 13 dogs is shown in Figure 1. The repeat occlusion data for one dog was not useable because of technical difficulties with the blood flow measurements. Mean blood flow to the myocardium supplied by immature collateral vessels increased significantly from 0.28 ± 0.17 to 0.37 ± 0.22 ml/min per g from occlusion 1 to occlusion 2 (P = 0.01). Flow increased significantly to each layer (P = 0.04). However, the magnitude of this increase to each of the four layers was not statistically different (P = 0.58). The endo/epi increased significantly: 0.23 ± 0.15 to 0.32 ± 0.17 (P = 0.01). Hemodynamic data were not different during each of the occlusions (see Table 1). Mean flow to the noncollateral-dependent region was 1.12 ± 0.65 ml/min per g during occlusion 1 and 1.12 ± 0.21 ml/min per g during occlusion 2.

After 2 hours, repeat occlusion in 10 dogs demonstrated that mean blood flow and the endo/epi were no different from that of occlusion 1 of the first set of occlusions indicating that vascular reactivity had returned to baseline (Fig. 1). Hemodynamic measurements during these occlusions were similar (see Table 1).

The effect of administration of nitroglycerin in 10 dogs is shown also in Figure 1. Using pre-nitro-
Occlusion 2
Occlusion 1
Control

There are no significant differences between the aortic pressures or between the mean left atrial pressures.

**TABLE 1**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>n</th>
<th>Heart rate (beats/min)</th>
<th>Aortic pressure (mm Hg)</th>
<th>Mean left atrial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>80 ± 21*</td>
<td>118 ± 10</td>
<td>77 ± 7</td>
</tr>
<tr>
<td>Occlusion 1</td>
<td>13</td>
<td>114 ± 27</td>
<td>117 ± 15</td>
<td>81 ± 13</td>
</tr>
<tr>
<td>Occlusion 2</td>
<td>13</td>
<td>110 ± 29</td>
<td>115 ± 15</td>
<td>81 ± 14</td>
</tr>
<tr>
<td>Pre-nitroglycerin</td>
<td>10</td>
<td>103 ± 20</td>
<td>118 ± 11</td>
<td>88 ± 10</td>
</tr>
<tr>
<td>Post-nitroglycerin occlusion</td>
<td>10</td>
<td>85 ± 18</td>
<td>117 ± 13</td>
<td>85 ± 12</td>
</tr>
</tbody>
</table>

Hemodynamic data obtained at the time blood flow measurements were carried out during: the control state; two left circumflex occlusions separated by 15 minutes (1 and 2); two left circumflex artery occlusions separated by 15 minutes before and after nitroglycerin administration. *n = number of dogs. Data are mean ± 1 SD.

* Control heart rate is different from all occlusion heart rates except post-nitroglycerin (P = 0.004). There are no significant differences between the aortic pressures or between the mean left atrial pressures.

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**FIGURE 1.** Myocardial blood flow to the collateral-dependent region measured during two sets of circumflex occlusions: the first two occlusions performed in 13 dogs were separated by 15 minutes (opened and closed circles); the second two occlusions performed in 10 dogs 2 hours later were separated by 15 minutes and in nitroglycerin administration (opened and closed triangles). Only the mean values for each transmural layer are given. Mean flow and endo/epi values are mean ± SD.

glycerin flow as the control, mean flow in the collateral-dependent region was found to increase significantly from 0.28 ± 0.20 to 0.46 ± 0.32 ml/min per g (P = 0.01). Flow to layers 2, 3, and 4 increased significantly (P = 0.01). Flow to layer 1 was 0.49 ± 0.25 ml/min per g during pre-nitroglycerin occlusion and 0.53 ± 0.27 ml/min per g during post-nitroglycerin occlusion, the increase not being statistically significant (P = 0.63). The endo/epi increased significantly from 0.25 ± 0.14 to 0.58 ± 0.27 (P = 0.01). In order to determine whether nitroglycerin produces an independent effect, flow measured during occlusion 2 of the first occlusion set was used as the control. The change in mean flow tended to be greater but did not reach statistical significance (P = 0.08). However, increase in flow to transmural layers 2, 3, and 4 following nitroglycerin was significantly greater than with repeated occlusion alone (P = 0.04). The endo/epi increased significantly more (P = 0.01) between pre- and post-nitroglycerin occlusions, compared with the first set of occlusions. In the normally perfused region, mean flow decreased significantly from 1.06 ± 0.33 ml/min per g during pre-nitroglycerin to 0.82 ± 0.34 ml/min per g during the post-nitroglycerin occlusion (P = 0.01), while the endo/epi in this region remained the same. Hemodynamic measurements are listed in Table 1.

Two dogs were studied in the reverse order (performing the nitroglycerin set of occlusions first), and no difference was found between responses of these dogs and the others.

**Discussion**

The canine model developed in this investigation utilized a partial coronary stenosis to stimulate the development of collateral perfusion rapidly. The technique employed is similar to that described by Cohen and Yipintsoi (1979). Since flow can be acutely interrupted in the coronary vessel at the point of stenosis, the function of the collateral vessels during transiently augmented flow can be evaluated, i.e., flow measured in the collateral-dependent area is due to flow through collateral vessels. The posterior papillary region was most often selected, since it had the lowest flow of the regions perfused by the left circumflex coronary artery, and has been shown to have a high correlation between area at risk from circumflex occlusion and infarction area (Koyanagi et al., 1982). Flow to all layers in the ischemic region did increase significantly from day 7 to day 14, and from occlusion one to occlusion two, while nonischemic flows remained the same. The question arises whether the increase in blood flow to the collateral-dependent area after 7 days might be related to the preferential loss of microspheres. This has been described in infarcted but not non-infarcted areas of myocardium (Murdock and Cobb, 1980). Since the collateral-dependent region in the present study was not infarcted, the increase in flow to the collateral-dependent region is not likely to be a result of microsphere loss.

Total occlusion, reperfusion, and reocclusion after 15 minutes demonstrated clearly increased flow to all layers of collateral-dependent myocardium. Thus, some effect of the first occlusion stimulated further collateral flow during the subsequent occlusion. Hemodynamic parameters were similar during
both occlusions and, therefore, could not have been responsible for the flow increase.

That collaterals do not open immediately when stimulated was suggested by earlier studies of Greenfield et al. (1972). Following acute interruption of flow in a vein graft, reactive hyperemia was markedly attenuated as the time of occlusion increased. These observations suggested that existing collaterals on which the myocardium was dependent had begun to close after the insertion of the vein graft, and did not open immediately when this new source of blood supply was acutely interrupted. Information concerning the time course of this phenomenon can be obtained from the pre-nitroglycerin occlusion performed 2 hours after release of the second occlusion and reperfusion. Hemodynamics were similar during the second and third occlusions, and mean flow and endo/epi were not statistically different. Thus, in 2 hours, the immature collateral vessels had returned to baseline reactivity.

Nitroglycerin improves indices of coronary collateral function when the vessels are well developed (Fam and McGregor, 1964; Cohen et al., 1973; Capurro et al., 1977). The literature remains controversial concerning the effect of nitroglycerin on collateral vessels in the innate, unstimulated state (Cohen et al., 1973; Bache et al., 1975; Becker, 1976). When the amount of blood flow to an ischemic area of myocardium is maintained at a constant level, the effect of nitroglycerin is to redistribute flow toward the endocardial layer (Swain et al., 1979). The data from the present study indicate significant redistribution of flow, as well as a significant increase in flow to layers 2, 3, and 4 in the collateral region during occlusion following nitroglycerin administration.

The nitroglycerin data, viewed independently, would imply a marked effect of nitroglycerin on the blood flow to a region of myocardium supplied by immature collateral vessels. These results, however, must be interpreted within the context of the prior occlusion, reperfusion, and reocclusion data. When compared with increases in collateral blood flow due to repeated occlusion alone, the increases due to nitroglycerin become less dramatic: in fact, the increase in mean flow was not statistically significant. Thus, investigations which utilize repeated occlusions to test the effects of drugs or other interventions on blood flow to an area of myocardium supplied by immature collateral vessels must be viewed within the context of these data.

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Address for reprints: Joseph C. Greenfield, Jr., M.D., Box 3246, Duke University Medical Center, Durham, North Carolina, 27710. Received March 19, 1983; accepted for publication December 1, 1983.

References


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