Changes in Coronary and Collateral Flows and Adequacy of Perfusion in the Dog following One and Three Months of Circumflex Occlusion

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SUMMARY. We investigated changes in circumflex, left anterior descending (LAD), and right coronary artery flows as well as changes in collateral flows to these vessels after long-term circumflex occlusion. Coronary and collateral flows of each vessel were determined simultaneously in an isolated heart preparation in which the vasculature was maximally diluted with dipyridamole. The resistances as related to total heart weight of the circumflex, LAD, and right coronary arteries of 16 control dogs were found to be 0.59 ± 0.06, 0.93 ± 0.09, and 2.37 ± 0.17 (mean ± SEM) mm Hg/[(ml/min)/100 g], respectively. Total minimal coronary resistance was 0.21 ± 0.01. In 10 dogs subjected to occlusion for 1 month no significant change in circumflex coronary resistance was observed, but the resistance of the unimpaired vessels decreased significantly. The resistances of the LAD and right coronary arteries were 0.66 ± 0.04 and 1.72 ± 0.13, respectively. Both values were considerably less (P < 0.01) than control. In nine dogs subjected to occlusion for 3 months the resistance of the unimpaired LAD and right arteries, as well as the circumflex coronary resistance, were not significantly different from control. We also found that retrograde flows for all vessels increased 7-fold after 1 month and 10.5-fold (relative to control) after 3 months of occlusion. From these data we conclude that vascular adaptations, which occurred in response to an ischemic stimulus, are responsible for the long-term regulation of the metabolic needs of the myocardium.

IT HAS BEEN established by many investigators that coronary collateral vessels develop in the face of coronary occlusion in an attempt to supply the metabolic needs of the myocardium. The adequacy of this supply certainly determines the long-term survival of the underperfused heart muscle and its ability to maintain the circulation. Although much is known about the collateral circulation, the long-term role of the major coronary vessels during the period of collateral development and the mechanisms governing vascular adaptations to an ischemic stimulus are poorly understood.

In an earlier study from this laboratory tentatively suggested that gradual coronary occlusion not only produces changes in collateral vascularization but leads to changes in the peripheral coronary vasculature. The purpose of this study was twofold. The flow changes were determined, with a refinement of earlier techniques, in both the coronary and collateral circulation after gradual occlusion of the circumflex coronary artery over a period of several months. The interrelationships of vascular changes between the coronary and collateral beds were assessed. The questions addressed

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Methods

Thirty-five young adult male mongrel dogs weighing between 15 and 18 kg were used in this study. All dogs had to have a normal electrocardiogram (leads I, II, III) and negative tests for microfilariae before they were accepted into a 3-week conditioning program consisting of treatment for intestinal parasites and vaccinations for distemper and hepatitis. The coronary and collateral flows were determined for 16 control and 19 experimental dogs in an isolated heart preparation. In the latter group an Ameroid occluder was placed on the left circumflex coronary artery of 10 dogs for 30 days and 9 dogs for 90 days.

SURGICAL PLACEMENT OF AMEROID CONSTRUCTOR

The experimental dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv). We used sterile procedures to enter the chest at the 5th intercostal space while ventilation was maintained with a Bird Mark 7 respirator. The pericardium was cut from the base to the apex of the heart, and the circumflex coronary artery was isolated just distal to
the main left coronary artery. The latter procedure had to be performed bloodlessly and with a minimum of tissue trauma to avoid widespread adhesions. An Ameroid occluder with an inner diameter of 2.77 mm was placed around the circumflex artery during continuous monitoring of lead I1 of the electrocardiograph. The electrocardiograph was observed for several minutes after placement of the Ameroid occluder to ensure that premature closure of the vessel had not occurred as a result of improper placement. The heart was lavaged with saline which was removed by suction before the pericardium was closed. The ribs were approximated with two strands of stainless steel wire and the chest was closed in a routine manner. A chest drainage tube was inserted through the 7th intercostal space and connected to a water seal. As the dog began to recover from the anesthetic, the chest tube was removed and a dose of 30 mg of pentazocine (Talwin) was administered as an analgesic.

**ISOlATED HEART PREPARATION**

The circumflex, right, and anterior descending coronary vessels were cannulated and perfused simultaneously in an isolated heart preparation, which has been described in detail in previous publications. In the experimental dogs the circumflex artery was cannulated just distal to the occluder. Dipyridamole was injected into the blood perfusion reservoir in the amount of 5.0–10.0 mg/700 ml of blood volume to obtain maximum vasodilation. A pressure-flow curve was obtained by lowering the blood reservoir pressure to 30, 40, 60, and 80 mm Hg for 3–4 seconds and recording phasic coronary flows with three Statham SP 2202 flowmeters. Between each decrease in pressure, the perfusion pressure was returned to 100 mm Hg to allow control flows to be reestablished. A pressure-flow curve was plotted using the late diastolic values for flow (Fig. 1, panel A) and pressures which were corrected for pressure losses in the perfusion system. The vascular resistances were obtained from the slope of the corrected pressure-flow curve and adjusted for small variations in hematocrit of the perfusate so that all values were standardized to a hematocrit of 40 vol %.

The retrograde flow method was used to obtain an index of coronary collateral flow in the right, circumflex, and anterior descending coronary arteries. Since collateral flows also are affected by the perfusion pressure, retrograde flows were corrected for pressure losses introduced by the perfusion system. After termination of the experiment, the perfusion system was calibrated for blood flows, and the hematocrit and heart weight were recorded.

**DATA ANALYSIS**

All analysis of data was performed in a manner identical to that previously described; however, a more automated computer-assisted approach was used. The three coronary flows and a time code signal (Datum model 7300) were simultaneously recorded on a Hewlett-Packard 7750 direct-writing strip chart recorder and Hewlett-Packard 3960 analog magnetic tape recorder. After an experimental procedure (for instance, a change in perfusion pressure), a time code section during the steady state flow response was obtained from the strip chart record and punched on IBM cards. These cards served as commands to a PDP-7 computer to digitize the data from the tape recorder during this time interval and store the information on a mass storage disk. The flow patterns were then displayed on an oscilloscope as shown in panel A of Figure 1. The two vertical lines (cursors) could be moved independently along the waveform and centered over the late diastolic flow section. A numerical integration was obtained for data points between the two cursors, and by use of the stored calibration information the late diastolic flow at the given pressure was printed by the computer. Panel B of Figure 1 illustrates how the average flow over several flow cycles was obtained. Late diastolic flow values could be determined simultaneously for all three coronary flow patterns as shown in panel C.

**Results**

**OBSERVATIONS ON CORONARY FLOW DISTRIBUTIONS**

By monitoring all three coronary flows simultaneously, the isolated heart preparation used in this study allowed blood flow redistributions among the coronary vessels to be observed following various manipulations. Figure 2 illustrates typical flow patterns in a heart which had been subjected to an Ameroid occlusion of the circumflex artery for 90 days. Control flows in the left anterior descending, circumflex, and right coronary arteries are shown on the left side of the record. A schematic representation of the perfusion preparation is shown in Figure 3. During control flow the valve at location 1 was open and the valve at location 2 was closed. Blood flow from the perfusion reservoir to the circumflex vessel was suddenly interrupted.
CONTROL CLAMP RETRO

Figure 2 Record of the flows of the left anterior descending (LAD), circumflex (CIRC), and right coronary arteries during control, clamp, and retrograde periods in a dog heart subjected to 3 months of circumflex occlusion.

(valve 1 closed) for 5 seconds, as indicated by the "clamp" period of Figure 2. A concomitant increase in flow in the LAD and right coronary arteries was observed, indicating that blood flow to the circumflex was maintained via collaterals to this vessel. The circumflex was then opened to atmospheric pressure (valve 2 of Figure 3 was opened) and blood flow was seen to be retrograde from the circumflex artery, as shown by the negative deflection on the record of Figure 2. A further increase in both LAD and right coronary flows was recorded during the "retro" period. An increased flow in the other two vessels during retrograde flow measurements was seen in 80% of 208 observations in dogs subjected to 1 month and 3 months of coronary occlusion. However, the magnitude of the retrograde flows in control hearts was so small that it was difficult to distinguish flow changes from experimental error in this group of experiments. Figure 2 also shows that maximum retrograde flow occurred during systole.

Effects of Gradual Occlusion on Retrograde Flows

After 1 month of Ameroid placement (total coronary occlusion occurs at around 17 days) retrograde flows measured for the right, LAD, and circumflex arteries were significantly increased in comparison to control values. Figure 4, a semilogarithmic plot of the retrograde flows vs. time, shows that the retrograde flows obtained for the right, LAD, and circumflex arteries increased from 1.76 ± 0.29, 8.76 ± 1.24, and 9.77 ± 1.31 (mean ± SEM) ml/min, respectively, to 11.4 ± 4.1, 65.6 ± 7.4, and 93.0 ± 10.6 ml/min, respectively. A nonpaired t-test showed the changes to be significant with respect to control ($P < 0.01, P < 0.001, P < 0.001$). In the group of dogs subjected to Ameroid occlusion for 3 months the respective retrograde flows were 18.3 ± 10.0, 102.1 ± 22 and 141.6 ± 19.2 ml/min and represented a significant increase from control ($P = 0.03, P < 0.001, P < 0.001$, respectively). Thus, during the 1st month retrograde flows increased almost 7-fold, and by 3 months a 10.5-fold increase from control was noted.

Effects of Gradual Occlusion on Coronary Resistances

The coronary resistances of the control, 1-month, and 3-month Ameroid groups are shown in Figure 5. The resistances of the circumflex, LAD, and right coronary arteries of the control dogs were, respectively, 0.59 ± 0.06, 0.93 ± 0.09, and 2.37 ± 0.17 resistance units, expressed in mm Hg/[(ml/min)/100 g]. The resistance of the circumflex...
Figure 5 A plot of coronary resistances with respect to time of Ameroid occlusion for the circumflex (CIRC), left anterior descending (LAD), and right coronary arteries. Resistances were those obtained after correction for pressure losses of the perfusion system and hematocrit, and based per 100g heart weight.

artery in the 1-month Ameroid group (0.6 ± 0.03 units) did not differ significantly from values for control dogs. However, the resistances of the unimpaired vessels were significantly less (P < 0.01) than the resistances in the control group. The resistances of the LAD and right coronary arteries were 0.66 ± 0.04 and 1.72 ± 0.13 units, respectively. In the 3-month Ameroid group the resistance of the circumflex was 0.73 ± 0.09 resistance units, a value which was not significantly different from that of the control group (P = 0.20). However, the resistances of the unimpaired LAD and right coronary arteries were greater relative to the 1-month occlusion group (0.95 ± 0.08 and 2.0 ± 0.2, respectively). Thus, the resistances of the unimpaired vessels following 3 months of occlusion were not significantly different from the control group (P = 0.85 for LAD and P = 0.20 for the right coronary artery).

Comparisons of Antegrade and Retrograde Flows

Since coronary antegrade and retrograde flows were measured in the same heart in these studies, it was of interest to determine whether the retrograde flow in a particular vessel could quantitatively approach the magnitude of the antegrade flow. The ratio of retrograde to antegrade flow was determined for each vessel for every experiment as a function of time, as shown in the semilogarithmic plot of Figure 6. The average ratios for the control dogs for the right, circumflex, and LAD were 0.02 ± 0.004, 0.03 ± 0.005, and 0.05 ± 0.007 (mean ± SEM), respectively. After 1 month of circumflex occlusion these ratios were 0.11 ± 0.04, 0.34 ± 0.03, and 0.27 ± 0.04, respectively. After 3 months the ratios for the circumflex reached a value of 0.94 ± 0.11, and the LAD was 0.99 ± 0.27. The retrograde flows in the right coronary artery at this time also had increased; however, the increase was not as dramatic as for the left coronary vessels (ratio = 0.3 ± 0.13). From these data it could be concluded that following 3 months of Ameroid placement, above resting circumflex and LAD antegrade flows could be sustained by collateral vessels. Figure 6 also illustrates that there seems to be preferential collateral formation between the dominant coronary vessels.

Discussion

The results of this study indicate that gradual constriction of the left circumflex coronary artery produces changes in both the coronary and collateral vasculature over a time span of several months. Following the first month of occlusion the coronary flows in the unimpaired vessels increased significantly, but by 3 months they were not significantly different from control values. No significant change in the resistance of the circumflex was noted during the same period of time but a continuous increase in collateral flow occurred. The determination in the same heart of coronary antegrade and retrograde flows provided additional quantitative information about the adequacy of coronary perfusion with collateral development.

Since these studies were carried out on a maximally dilated vascular preparation, changes in vascular tone in these studies could be ruled out. The isolated heart preparation allowed most extrinsic factors, such as pressure, nervous influences, blood gas values, pH, and the load on the heart to be controlled. Thus, in the present study the changes
in coronary flow are due to changes in the vascularity of the various coronary beds.

This study confirms the observations by many investigators that coronary occlusion stimulates collateral formation and increases collateral flow.1, 2 The record of Figure 2 and many observations have shown that coronary flows in the other two vessels increase when one vessel is "clamped" and that a further increase in coronary flows occurs during the retrograde flow measurements. This observation leads to the conclusion that during the "clamp" period collateral flow may be limited by the resistance of the distal vascular bed. The rise in coronary flows during the retrograde flow measurement then seems to indicate that retrograde flow, at least in a heart with well developed collaterals, overestimates actual collateral flow.3, 4 However, retrograde flow may be an index of the maximum flow that can be delivered by collateral vessels. Figure 2 also illustrates clearly that the retrograde flow measured for a single vessel consists of the sum of the flow contributions from the other vessels.

The data from this study indicate that collateral development is rather extensive and that retrograde flows can quantitatively approach the magnitude of antegrade flow. This means that collateral resistance approaches minimal coronary resistance. Since collaterals are connected in series with the coronary vessel, their combined resistances would allow only 50% of maximum coronary flow. Thus, after 3 months of coronary occlusion, coronary reserve is not established completely. In a concurrent study (unpublished) in which Ameroid occluders were implanted in an identical manner, histopathological studies revealed various degrees and extents of myocardial infarction. In the latter study five control and five sham-operated hearts were compared with eight hearts on which an occluder had been placed around the circumflex for 17, 30, and 40 days. Whole heart transverse sections were stained with fuchsins, hematoxylin and eosin, and Movat's pentachrome. Movat's stain was particularly useful in delineating areas of myocardial infarction. Some degree of infarction was observed in all hearts subjected to circumflex occlusion, and this was located predominantly in the posteromedial and posterolateral aspects of the left ventricle.

In a number of recent studies the effects of several hours of acute coronary occlusion on the distal vascular bed have been investigated. Following the release of the acute occlusion, coronary reflow was decreased because of an elevated coronary resistance.10-12 It has been shown that this increased vascular resistance is the result of swelling of injured myocardial cells which have lost their capacity for volume regulation.12 It is doubtful, however, that the acute increase in vascular resistance would have been sustained over a long period of time because an injured cell would either recover or necrose. In the present study "reflow" to the gradually occluded circumflex coronary artery occurred during a prolonged period of time to collateral vessels which could have either prevented cellular injury or allowed the vascular bed to stabilize according to the above-mentioned mechanism. This may explain our observations that the coronary resistance, distal to the occlusion, was not significantly different from control following 1 month and 3 months of Ameroid occlusion.

To interpret the results of this study with regard to the observation that the resistance of the unimpaired coronary vessels was decreased during the 1st month of circumflex occlusion, it could be postulated that the regional hypoxic stimulus, emanating from the impaired vessel and its surrounding myocardium, led to the development of both intra- and intercoronary collateral formation. In other words, the unimpaired vessels were initially called upon to perfuse a larger mass of the myocardium. With the development of collaterals to the impaired circumflex bed this stimulus gradually subsided and after 3 months of occlusion the intercoronary collateral network was so well developed that the requirement for circumflex perfusion was almost totally satisfied, as shown in Figure 6. Thus, with the loss of the regional ischemic gradient the unimpaired vessels no longer were required to perfuse an increased myocardial mass. Since it has been shown that the removal of the ischemic stimulus can result in regression of coronary vessels,13, 14 this mechanism could have been responsible for the return of the resistances of the unimpaired vessels toward control values (Fig. 5).

The present study, as well as our previous work,1 seems to suggest that changes in the vascular pattern of the coronary circulation are governed primarily by ischemic stimuli. Vascular growth seems to occur along ischemic gradients, which may exist either between epicardial and endocardial layers of the heart4 or, as in this study, between one region of the myocardium and another. Thus vascular growth can be viewed as a long-term regulatory mechanism in response to an ischemic stimulus.

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