Progressive Perfusion Impairment during Prolonged Low Flow Myocardial Ischemia in Dogs

LAWRENCE H. FRAME, M.D., AND WM. JOHN POWELL, JR., M.D.

SUMMARY Recent studies have shown that after total coronary artery occlusion, there is impaired "reflow" of blood accompanied by myocardial and capillary endothelial cell swelling. To investigate the effect of prolonged low flow myocardial ischemia on coronary vascular resistance, regional hyperperfusion of the distal left anterior descending coronary artery was studied in 31 unanesthetized dogs. Under hypothermic conditions (33-34°C) in 17 dogs there was a consistent progressive increase in distal left anterior descending coronary artery vascular resistance starting at 90 minutes (median) after onset of ischemia. By 110-140 minutes ischemic antegrade flow decreased by 35 ± 4% (SEM) (P < 0.01). Directionally similar flow changes were observed in six euthemic experiments using the tetrodotoxin vasohib technique. Light microscopy did not reveal hemorrhage as a cause of the increased vascular resistance. The perfusion impairment did not occur in two euthemic, nonischemic hearts. In five dogs elevation of serum osmolality by 10-11 mOsmol/liter with mannitol attenuated the progressive increase in flow. Thus, a progressive perfusion defect exists in the ischemic low flow state in the heart which presumably contributes to the extent of eventual necrosis.

THE FACTORS leading to irreversible cell injury during myocardial ischemia are poorly understood. Significantly impaired reperfusion following total arterial occlusion has been demonstrated in the brain, and the kidney. In the heart this phenomenon also has been demonstrated during "reflow" of blood following total occlusion of a single coronary artery. Recent studies from our laboratory have demonstrated perfusion defects in the posterior papil-
lary muscles of dogs following temporary circumflex artery occlusion of either 40 or 60 minutes' duration. Associated electron microscopy revealed extensive myocardial and endothelial cell swelling. The infusion of hyperosmotic mannitol primarily during the reflow period dramatically reduced the amount of cell swelling and the extent of eventual necrosis.

In all of the above studies severe ischemia was produced by total arterial occlusion. Clinically, myocardial ischemia may involve a reduction rather than a total interruption of blood flow. It is not known whether a progressive perfusion impairment develops under these circumstances. Accordingly, studies were undertaken to assess the perfusion of ischemic myocardium during the low flow state. The experimental model allowed continuous monitoring of coronary perfusion and precise control of the associated hemodynamics. Specific attention was paid to the time course and extent of the perfusion impairment and to factors influencing this impairment.

**Methods**

Experiments were performed in 31 mongrel dogs of either sex, weighing 25–30 kg, and anesthetized with chloralose (60 mg/kg, iv) and urethane (600 mg/kg, iv). Tracheal intubation was performed and ventilation with 97% O$_2$-3% CO$_2$ was provided during the preparation period using a Harvard respirator. The blood from two or three donor dogs was used for each experiment. Each donor dog was anesthetized with methohexital (Brevital, Lilly) (25 ml of 1% solution in saline). Sodium bicarbonate (44 mEq) and heparin (6 ml; 1,000 U/ml) were added to the blood from each donor.

In the experimental dogs, after intravenous administration of 3–4 ml of heparin and intramuscular injection of procainamide (Pronestyl) 1,000 mg divided into three sites, the right heart bypass preparation was established as follows (Fig. 1): The inferior and superior venae cavae were cannulated and the azygos vein was divided. The caval return was directed through a bubble oxygenator to a reservoir and heat exchanger. Oxygenated blood was delivered by a calibrated pump to the pulmonary artery. The right heart was isolated by placing a tight ligature around the pulmonary artery distal to the first major branch. The second cannula was perfused by a system similar to that described (Fig. 1). The inferior and superior venae cavae were cannulated and the azygos vein was divided. The caval return was directed through a bubble oxygenator to a reservoir and heat exchanger. Oxygenated blood was delivered by a calibrated roller pump to the pulmonary artery. The right heart was isolated by placing a tight ligature around the pulmonary artery catheter. The right heart then received only coronary venous return which was led by siphon drainage to a level of between 10 and 20 cm below the heart. This level remained constant throughout each experiment. The rate of flow to the pulmonary artery determined left ventricular cardiac output. Adjustments in cardiac output permitted fine control of left ventricular end-diastolic pressure. A separate roller pump allowed withdrawal or infusion of oxygenated blood to or from the blood reservoir through catheters placed in both femoral arteries as needed to maintain a constant mean aortic pressure of 75 mm Hg.

Ischemia was produced in the area perfused by a small cannula, 8–10 cm in length, which was placed into the midportion of the left anterior descending coronary artery or one of its major branches. Flow through this cannula was delivered as follows: Oxygenated blood was withdrawn from the heat exchanger and infused into the coronary perfusion line by a Harvard dual syringe constant infusion pump (model 600–900). The cannula perfusion pressure was controlled by adjusting the height of a vertical overflow column connected to the coronary perfusion line near the heart. Cannula perfusion pressure was monitored near the heart beyond the overflow column. Cannula flow was measured every 4 minutes by subtracting the volume of overflow per unit of time from the constant infusion rate of the Harvard pump, which was calibrated daily. Two-minute flow calibrations at the infusion pump rate of 4.2 ml/min (which was used for all the low flow periods) were reproducible to within at least 0.05 ml/min. The temperature of the ischemic area was maintained at euthemic levels in 19 experiments by placing a warm water sleeve around the coronary perfusion line and a heat lamp over the heart (Table 1).

In nine experiments the warm water sleeve and the heat lamp over the heart were not employed; this allowed the temperature of the ischemic area to become hypothermic. In seven of these nine experiments the coronary cannula was placed in the last major branch of the left anterior descending coronary artery. The area surrounding the primary ischemic area also was separately perfused through a second cannula placed in the proximal left anterior descending coronary artery distal to the first major branch. The second cannula was perfused by a system similar to that described for the first cannula, and during ischemia its perfusion pressure was lowered to the same level as the coronary perfusion pressure of the first cannula. The function of the second cannula was to decrease the amount of collateral circulation to the primary ischemic area. Visible epicardial anastomoses between the left anterior descending coronary artery...
artery or its branches and other coronary arteries were ligated. In 21 experiments (two hypothermic and 19 euthermic), to permit a greater reduction in cannula flow during ischemia, the primary cannula was placed in the midportion of the left anterior descending coronary artery so that a larger area was perfused by this cannula. This area included one or two major branches as well as the distal left anterior descending coronary artery. In these experiments a second cannula was not used, but much more extensive efforts were made to identify and ligate potential epicardial collateral vessels.

Aortic arch pressure was measured through a subclavian artery catheter. A metal cannula inserted through the left ventricular apex had a Y-shaped connection to two transducers which measured full left ventricular pressure and left ventricular diastolic pressure for monitoring left ventricular end-diastolic pressure. The first derivative of left ventricular pressure (LV dp/dt) was generated from the full left ventricular pressure signal by a differentiating circuit with a time constant of 0.001 second and a cutoff of 160 cycles/sec. This circuit was calibrated at the end of each experiment by supplying several ramp inputs of known slope. All pressures were measured using Statham P23 Db pressure transducers. A lead II electrocardiogram (ECG), an epicardial ECG from the ischemic area, mean aortic pressure, left ventricular pressure, left ventricular end-diastolic pressure, LV dp/dt, and coronary perfusion pressure were recorded on a Hewlett Packard 7900 eight-channel direct-recording oscillograph. The sinoatrial node was crushed, and the heart was atrially paced at a constant rate (usually 150 beats/min) (Medtronic model 5837 A-V pulse generator).

Thirty minutes before beginning each experiment propranolol (Inderal), 0.5 mg/kg, and mecamylamine (Inversine), 5 mg/kg, were given intravenously. During each experiment a solution containing 5 mg of propranolol in 500 ml of 5% dextrose in water was delivered to the blood reservoir at 2 ml/min. This maintained adequate beta-adrenergic receptor blockade and blood glucose levels and prevented hemoconcentration due to evaporative water loss. The first derivative of left ventricular pressure signal by a differentiating circuit with a time constant of 0.001 second and a cutoff of 160 cycles/sec. This circuit was calibrated at the end of each experiment by supplying several ramp inputs of known slope. All pressures were measured using Statham P23 Db pressure transducers. A lead II electrocardiogram (ECG), an epicardial ECG from the ischemic area, mean aortic pressure, left ventricular pressure, left ventricular end-diastolic pressure, LV dp/dt, and coronary perfusion pressure were recorded on a Hewlett Packard 7900 eight-channel direct-recording oscillograph. The sinoatrial node was crushed, and the heart was atrially paced at a constant rate (usually 150 beats/min) (Medtronic model 5837 A-V pulse generator).

During each experiment arterial pH, Pco2, Po2, sodium, potassium, hematocrit, blood glucose, and total protein were measured each hour. Plasma potassium and sodium concentrations were determined using an Instrumentation Laboratories flame photometer (model 143). Blood Po2, Pco2, and pH determinations were made with a Radionator blood microsystem BMS-3 and acid-base analyzer PHM 71. The blood gas analyzer was calibrated with humidified gas of known partial pressure. The pH analyzer was calibrated by Precision buffer solution (Radiometer). The arterial Po2 was maintained above 100 mm Hg and Pco2 and pH were kept constant within a normal range. Plasma electrolytes and glucose were nearly constant during each experiment.

After autonomic blockade was induced cannula flow and systemic hemodynamics were monitored until all hemodynamic parameters were stable. Ischemia then was produced by lowering the cannula perfusion pressure sufficiently to reduce cannula flow to the desired fraction of control flow. During the first few minutes of ischemia, left ventricular end-diastolic pressure was permitted to rise slightly to levels no higher than 8 cm H2O above control and was then held constant throughout the experiment. Mean aortic pressure was always maintained at 75 mm Hg.

In six experiments the washout of radioactive 51Kr was used to measure total blood flow in the ischemic area. 51Kr (1 ml) in saline (1 mCi/ml) was injected rapidly into the ischemic area through the coronary perfusion cannula. After injection of 51Kr the line was quickly flushed with a volume of blood equal to that of the distal cannula. The rate of 51Kr washout was assessed using a Nuclear-Chicago counting apparatus (models 8725, 8735, 821330) mounted close to the external chest wall and aimed at the ischemic area. The number of counts per 12-second period was plotted on semilogarithmic paper. The flow per 100 g of tissue was calculated from the initial slope of this plot which was linear for 4-6 minutes.

An infusion of 20% mannitol was begun after the appearance of a perfusion impairment in five experiments. Mannitol was infused at 8.3 ml/min into the mixed venous blood before it passed through the oxygenator. Osmolarities from the pulmonary artery line were measured before the infusion and at 10 minute intervals thereafter using a wide range (model 3W) osmometer (Advanced Instruments). At the end of each experiment, two full-thickness core biopsies about 1 mm in diameter were taken from the center of the ischemic area of myocardium and two from the normal-appearing myocardium on the posterior wall perfused by the circumflex artery while the heart was still functioning in situ. The biopsies were obtained with a high speed drill driven by compressed air (Steel's trephine drill biopsy, Downs Surgical). Within 5-10 seconds the biopsy specimens were immersed in glutaraldehyde (2.5% glutaraldehyde in a phosphate buffer of 300 mOsml/liter at a pH of 7.4) at 4°C and stained with hematoxylin and eosin. Light microscopy revealed either no hemorrhage or small scattered foci of several extravasated red blood cells in each biopsy specimen. When the histological specimens were arranged randomly, it was not possible, without prior knowledge of the protocol for each slide, to identify which specimens had been subjected to ischemia on the basis of extravasated red blood cells. No thrombi were seen on microscopic examination.

### Table 1: Summary of Experimental Protocols

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<thead>
<tr>
<th>Hypothermia</th>
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<td>9 hypothermic low flow*</td>
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<tr>
<th>Euthermia</th>
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<tbody>
<tr>
<td>17 euthermic low flow†</td>
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<td>12 without mannitol infusion</td>
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<tr>
<td>5 with mannitol infusion</td>
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<td>2 euthermic control experiments without low flow</td>
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* One additional experiment was not included because of hemorrhage.
† Two additional experiments were not included because of severe arrhythmias.
To establish that a discrete area of ischemia had been produced and to estimate the size of this area, silicone rubber (Microfil, Canton Biomedical Products) infusions into the aortic root were employed in a manner similar to that described previously. The ischemic area perfused by the cannula comprised approximately 10–15% of the left ventricle, as determined by serial slicing of the heart.

Statistical significance was calculated using Student's t-test. Where confidence limits are indicated, they always refer to the standard error of the mean.

Results

HYPOTHERMIC EXPERIMENTS

In nine dogs the heating apparatus was not employed, so that the myocardial temperature in the area of low flow ischemia fell below euthermic levels (Table 1). In these experiments the epicardial temperature in the center of the distribution of the hypoperfused artery ranged between 33°C and 34°C, a value substantially below that of the normal mongrel dog rectal temperature of 39 ± 0.5°C. In our experience left ventricular cavity temperatures are 1°C higher than rectal temperatures. In these experiments the temperature of the heat exchanger was maintained at 37°C and the temperatures, measured in the left ventricular cavity with a thermistor probe, ranged between 36°C and 37°C.

The level of cannula blood flow when ischemia was produced by lowering the cannula perfusion pressure ranged from 4% to 40% of the control blood flow at a perfusion pressure of 75 mm Hg. The individual percentages in the nine experiments were: 4%, 6%, 7%, 12%, 14%, 15%, 20%, 25%, and 40%. In seven of these nine experiments no change in antegrade flow occurred under conditions of prolonged (140–210 minutes) low flow perfusion. Two of the experiments revealed decreases in cannula blood flow during the time of low flow perfusion. In one of these a 25% decrease in flow occurred suddenly at the time that an inadvertently large adjustment in the pumping rate into the pulmonary artery line was made. Subsequently, despite restoration of the appropriate pulmonary artery pumping rate, cannula blood flow remained at this reduced level. In the other experiment the early ischemic blood flow was 15% of the control flow, and a subsequent 40% reduction in flow developed gradually and without any obvious hemodynamic explanation.

The mean data for all nine of these hypothermic, low flow experiments are depicted in Figure 2. The mean flow for each period during the experiment was normalized by dividing by the mean flow during the early ischemic period. Thus, the flow during early ischemia is by definition 1.0, and values less than 1.0 represent a decrease in blood flow compared with the early ischemic period. As can be seen, the cannula flow did not change significantly although, because of the above two experiments, there was a slight decrease in the mean level of flow in the last two time periods on the right of Figure 2. The two hypothermic experiments that were most similar to the euthermic ones, in that the single cannula perfusion system, extensive ligation of collaterals, and an initial ischemic flow between 4% and 6% of control flow were used, are among the seven that showed no change in cannula flow during prolonged ischemia.

EUTHERMIC EXPERIMENTS

In 19 dogs the epicardial temperature, monitored each 30 minutes in the center of the ischemic area, was maintained in the range of 37–40°C by the use of a heat lamp (or by closing the chest), and a warming jacket around the left anterior descending coronary artery perfusion line. In these animals the left ventricular cavity temperatures monitored with a thermistor probe ranged between 37°C and 39°C. Throughout any one experiment the temperatures were constant to within 1°C.

In 16 of the 17 euthermic experiments (Table 1) in which the blood flow to the distal left anterior descending coronary artery was reduced and maintained at a low level, a progressive further reduction in blood flow occurred during the period of ischemia. In these experiments the antegrade blood flow was lowered initially to between 3% and 7% of the control level at a perfusion pressure of 75 mm Hg. The hemodynamic data from a representative experiment are shown in Figure 3. The lowering of the coronary perfusion pressure from 75 to 21 mm Hg was associated with a substantial decrease in antegrade coronary blood flow to the distribution of the distal left anterior descending coronary artery from 12 ml/min to approximately 0.6 ml/min. The blood flow remained stable at this level for approximately 90 minutes and then progressively decreased toward zero. Note that the associated hemodynamics in the upper panels were maintained constant. The numbers labeled TBF *Kr represent values for the total blood flow to the ischemic area in ml/min per 100 g, as determined by the **Kr washout technique. Note that as the antegrade cannula flow fell, the total blood flow to the ischemic area also decreased.
PERFUSION IMPAIRMENT DURING ISCHEMIA/Frame and Powell

CANNULA FLOW (ml/min) 100 200 300 MINUTES 0 120 180 240

Figure 3  Hemodynamic data from a representative euthermic experiment. Note that cannula flow fell with the initial reduction in cannula perfusion pressure (CPP) and that there was a subsequent progressive decrease in cannula flow beginning after approximately 90 minutes of ischemia. Also note that there was a corresponding decrease in total blood flow as measured by radioactive "Kr washout (TBF "Kr). Other hemodynamic variables were held nearly constant during ischemia. MAP = mean aortic pressure; peak LV dp/dt = peak first derivative of left ventricular pressure; LVEDP = left ventricular end-diastolic pressure; CO = cardiac output.

The hatched bars in Figure 2 represent the combined cannula flow data for the 17 euthermic low flow experiments. In most experiments the cannula flow at a constant low perfusion pressure began to decrease between 80 and 110 minutes after the onset of reduced blood flow. After 110 minutes of constant low perfusion pressure, there was a continued further reduction in flow which was significant. There was variability in the rate of fall of cannula flow, but on the average blood flow fell to one-half its initial level by 30 minutes after the onset of the progressive decline in cannula flow.

The average blood flow values for the euthermic experiments in Figure 2 include four experiments that were not associated with a reduction in cannula flow during the period represented. Three of these experiments later showed reductions in coronary blood flow which was significant. There was variability in the rate of fall of cannula flow, but on the average blood flow fell to one-half its initial level by 30 minutes after the onset of the progressive decline in cannula flow.

The onset of progressive perfusion impairment (min)

ONSET OF PROGRESSIVE PERFUSION IMPAIRMENT (min)

The time from the beginning of ischemia to the onset of the progressive decrease in cannula flow in 16 euthermic experiments. The open circle represents an experiment discussed in the text which was not euthermic until late in the ischemic period.

antegrade flow began to decline. In the fourth experiment the temperature of the ischemic area was maintained between 35°C and 37.5°C, and no reduction in antegrade blood flow was noted during 175 minutes of low flow ischemia.

In two of the 19 euthermic dogs (Table 1), nonischemic control experiments were performed. In these experiments the preparation was identical to that in the euthermic ischemia experiments except that instead of producing ischemia by lowering the cannula perfusion pressure, cannula flow was monitored at a perfusion pressure of 75 mm Hg for at least 3 hours. In these two experiments in which the epicardial temperature in the area perfused by the cannula and the intracavitary blood temperature were 39°C and 41°C, respectively, there was no decline in antegrade blood flow.

In six of the above 17 low flow experiments, the total nutrient blood flow to the ischemic area was evaluated by serial measurements of the washout of "Kr in addition to the measurements of cannula blood flow. The data from each of these experiments are shown in Figure 5. The total nutrient blood flow was determined during the control period when the coronary perfusion pressure was 75 mm Hg, and between three and five times during the period of low flow ischemia. With the onset of ischemia, total nutrient flow was diminished to 19 ± 2% of control. When the total nutrient flow was measured at 20 minutes and 70 minutes of low flow ischemia, there was a consistent increase in total flow between 20 and 70 minutes averaging +22 ± 2% (P < 0.005) at a time when cannula flow was constant. In the experiment discussed above with the late onset of the fall in cannula flow (as represented in Fig. 4 by an open circle), there was a progressive increase in total nutrient flow to the ischemic area between 20 and 226 minutes of low flow ischemia (open triangles in Fig. 5). Because cannula flow remained nearly constant during this period, these findings reflect an initial increase in collateral blood flow. In each

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experiment, however, there was a subsequent decrease in the total nutrient blood flow to the region of ischemia. This decrease ranged from 12% to 62% (mean decrease, 24 ± 8%; P < 0.05) of the highest value noted during the low perfusion period. The corresponding mean fall in cannula flow was 58 ± 10%. It can be seen that there was some variation in the time at which total nutrient flow declined. This variation can be accounted for by the variation in the onset of the decrease in antegrade cannula flow. In every case the reduction in total nutrient flow, assessed by **Kr washout, occurred during the decrease in cannula flow.

**MANNITOL INFUSION**

In five of the 17 euthermic flow experiments, hyperosmotic mannitol was infused into the pump oxygenator reservoir beginning 30-35 minutes after the onset of the progressive decrease in cannula flow. The infusion of 8.3 ml/min resulted in mean increases in serum osmolality of 22 ± 8 mOsm, 16 ± 2 mOsm, 16 ± 5 mOsm, and 23 ± 5 mOsm above the control level of 326 ± 5 mOsm at 10, 20, 30, and 40 minutes of infusion, respectively. The mannitol infusion was begun at a time when the cannula blood flow had fallen to 37 ± 6% (SEM) of the initial ischemic flow. After 30 minutes of mannitol administration the hematocrit in these five experiments fell from 34 ± 2% to 29 ± 2% (P < 0.01).

Figure 6 shows the effect of 40 minutes of mannitol administration on the decrease in antegrade blood flow. The control ischemia data are from hearts not receiving mannitol at corresponding times of ischemia. Without the administration of mannitol, there was a continued progressive decline in antegrade blood flow which was significant at each of the times studied. With the administration of mannitol there were small decreases in mean blood flow which were not significant statistically. In one of the five experiments in which mannitol was administered, the **Kr washout technique was used to assess the total blood flow to the ischemic area just before the mannitol infusion and after 46 minutes of the infusion. In this experiment the infusion of mannitol increased blood flow from 15 to 18.7 ml/min per 100 g of cardiac muscle with an associated increase in osmolality to 36 mOsm above control, and a decrease in hematocrit from 30% to 26%.

**Discussion**

These data demonstrate that under hemodynamically controlled conditions an increased resistance to blood flow develops during myocardial ischemia. This perfusion impairment was demonstrated in a situation in which ischemia was severe but blood flow was not totally arrested. The perfusion impairment occurred only after a prolonged period of severe ischemia. Once it appeared, resistance to flow through the ischemic myocardium progressively increased.

The implication of these data is that during severe myocardial ischemia there is a self-perpetuating exacerbation of the degree of ischemia. Thus, following an initial decrease in blood flow, it is likely that the process leading to cell injury involves more than just a time-related deterioration of biochemical function and structure that would occur at a fixed low blood flow. Presumably, the progressive increase in ischemia leads to an acceleration of the development of eventual myocardial necrosis.

The total blood flow to an ischemic area consists of both impaired antegrade flow through the major artery to the area and collateral flow from other arteries. The contribution of collateral flow may be substantial. In the present study, whenever examined, total blood flow to the region of ischemia as measured by the **Kr washout technique, always decreased in association with the reduction in antegrade flow. This ruled out the possibility that the decline of cannula flow merely reflected a disproportionate increase in collateral flow. The relative driving pressures of antegrade flow and of collateral flow may be important in determining changes in the relative contributions of the two systems to
total flow as resistance increases at a microvascular level. In our experiments the controlled very low cannula perfusion pressure may explain why the antegrade flow decreased proportionately more than the total blood flow to the ischemic area.

The possibility that the progressive decrease in blood flow to the ischemic area was secondary to left ventricular hemodynamic events rather than to a primary process in the coronary vasculature was unlikely in the present experiments. The influence of left ventricular end-diastolic pressure on myocardial vascular resistance and of mean aortic pressure on collateral perfusion pressure was minimized by holding these pressures constant. Since left ventricular end-diastolic pressure, mean aortic pressure (through the tension-time index), heart rate, and LV dp/dt reflect major correlates of myocardial oxygen consumption and were constant or nearly constant, the metabolic influence of these variables on coronary vascular resistance was minimized. Changes in cardiac output were minimal and did not account for the perfusion impairment.

Thus, it is apparent that a progressive increase in resistance to perfusion, which was independent of associated hemodynamic changes, developed in the low flow euthermic experiments. This change in resistance to blood flow cannot be attributed to a reflex vasoconstriction mediated by the autonomic nervous system or to withdrawal of circulating catecholamines active at $\beta$-adrenergic receptors because effective ganglionic and $\beta$-adrenergic receptor blockade was maintained. It cannot result from humoral mediators released elsewhere in the body as a result of hypotension or other concomitants of cardiac failure because the systemic hemodynamics were maintained constant at physiological levels. It is unlikely that there was mechanical interference as a result of the experimental technique. For example, if obstruction of lymphatic flow by the ligation of collateral vessels or if the release of plasticizer from the cannula contributed importantly, increasing vascular resistance should have been observed in the hypothemic as well as the euthermic experiments. Thrombosis, perhaps related to temperature, is an unlikely mechanism, since the dogs were well heparinized and no thrombi were seen on microscopic examination. Furthermore, the two euthermic nonischemic control experiments were not associated with an increase in vascular resistance.

Extravasated red blood cells were seen in some of the tissue biopsies from the ischemic area. This raised the possibility that the impaired perfusion was due to hemorrhage with compression of the microvasculature. However, the impaired flow occurred in experiments in which there was no gross or microscopic evidence of hemorrhage, and when small numbers of extravasated red blood cells were present, there was no correlation between the extent of red cell extravasation and the degree of perfusion impairment.

The development of the perfusion impairment appears to be closely correlated with the severity of ischemia. The severity of ischemia depends not only on substrate delivery and the washout of metabolites but also on the level of metabolic activity of the tissue. Hypothermia, through its effect on cell metabolism, would be expected to decrease ischemia. In the present hypothermic experiments when blood flow was reduced, the perfusion defect did not develop. Thus, it appears that there may be an important relationship between the perfusion defect and myocardial metabolism.

A possible mechanism to explain the increasing resistance to blood flow during ischemia is suggested by data from recent histological studies. Cell swelling has been documented to occur with reflow of blood following total arterial occlusion in the brain and the heart. Our recent work has demonstrated that swelling of endothelial and myocardial cells occurs during and following total interruption of blood flow in the heart. This cell swelling may, through compression of microvasculature, contribute to impaired reflow of blood following release of vascular occlusion. Consistent with this hypothesis is the evidence that hyperosmotic mannitol, which remains extracellular and restores cell volume through an osmotic effect, improves reperfusion following arterial occlusion in the kidney, the brain, and the heart. In association with the reduction in cell swelling, there is a striking reduction in the extent of eventual myocardial cell necrosis.

In the present study with severe low flow ischemia, swelling of cellular elements may have occurred and contributed to the progressive perfusion impairment. However, this remains to be documented histologically. In this regard it was of interest that hyperosmotic mannitol attenuated the progressive decrease in blood flow. This effect of mannitol may have resulted from a nonspecific vasodilating action of this agent or from a possible decrease in blood viscosity due to the small decrease in hematocrit. However, the hypothesis that hyperosmolar mannitol slowed the progressive perfusion impairment through a reduction of ischemic cell swelling deserves further investigation. Since hyperosmolar mannitol slows the progressive perfusion impairment, it also may exert a salutary effect in terms of eventual salvage of myocardial cells in the low flow state.

Our previous study showed that a significant proportion of myocardial cells can be salvaged with the administration of hyperosmolar mannitol after an hour of total deprivation of blood flow. In our present experiments the ischemia probably was considerably less severe than during total cessation of blood flow because the total blood flow to the ischemic area ($^{13}$K$^+$ washout) remained at a level which was approximately one fifth that of the control nonischemic flow. Thus, it is likely in the present study that the progressive perfusion impairment began at a time of potential salvageability of a substantial proportion of the ischemic myocardial cells.

It is probable that myocardial ischemia in patients frequently involves a state of reduced but not totally arrested blood flow. The possibility that clinical myocardial ischemia involves a progressive perfusion impairment that exacerbates the degree of ischemia warrants investigation focused on the mechanism and the therapeutic potential of this phenomenon. In the present experimental study under controlled hemodynamic conditions, the development of this progressive impairment of coronary perfusion during low flow myocardial ischemia has been documented.

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References

Myocardial Tissue Recruitment in the Dog as Determined by Double Tracer Dilution Method

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With the technical assistance of Lynda Christie

SUMMARY The amount of tissue perfused, as determined from the difference in volume of distribution between a diffusible indicator (I4C-antipyrine) and an intravascular indicator (I25I-albumin) was measured at different values of coronary flow, perfusion pressure, and vasomotor tone in the working left ventricle of an open-chest dog. Coronary pressure and flow were regulated independently from the systemic circulation and coronary vasomotor tone was reduced by dipyridamole. At each flow vasomotor tone was assessed by using as a reference the maximal vasodilation induced by arrest of flow. Measured tissue space was considered to be related to the capillary surface area available for tracer diffusion and therefore to the number of perfused capillaries per volume of muscle. A relationship between coronary blood flow and tissue volume was observed. It was found to be independent of vasomotor tone. Vasodilation was found to increase available exchanging capillary surface at a constant perfusion pressure.

THE PRESSURE-VOLUME characteristics of the left coronary vascular system have been measured but it has not been determined whether the observed increase in vascular volume that follows an increase of coronary flow and perfusion pressure is the consequence of vascular distention, recruitment of closed vessels, or a combination of both mechanisms. We reasoned that if distention were the primary mechanism, and we measured tissue volume, it should remain constant as coronary flow increased. If recruitment of closed vessels occurred, tissue volume should increase and then reach a plateau when all vessels were perfused. By use of the double indicator technique described by Chinard et al., we measured the extravascular tracer distribution space of the canine left coronary system through a range of pressures and flows with autoregulation both intact and absent. Our data suggest that recruitment does occur throughout the physiological range of pressure and flow, and that autoregulation is an important factor in the control of the number of tissue units perfused.

Methods

Seven mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) and ventilated with a Harvard respirator. A double-lumen steel cannula provided with two crossing wire loops projecting 3 mm from the tip was passed via the carotid artery into the left main coronary artery. The purpose of the wire loops was to prevent the cannula from occluding either the circumflex or anterior descending branches. A bypass circuit took blood from the aorta into a thermostatted reservoir (37°C) from which it was pumped by a calibrated roller pump (Sarns model 3500) into one lumen.
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