Effect of Ischemia and Antianginal Drugs on the Distribution of Radioactive Microspheres in the Canine Left Ventricle

By Lewis C. Becker, Nicholas J. Fortuin, and Bertram Pitt

ABSTRACT
Radioactive microspheres were used to estimate the changes in regional myocardial blood flow occurring during acute myocardial ischemia. Carbonized 15-μm spheres were injected into the left atrium of 28 open-chest dogs and the radioactivity of selected areas determined after sacrifice. Acute occlusion of the left circumflex coronary artery produced a significant diminution in the proportion of microspheres reaching the circumflex area. In addition, there was a disproportionate decrease in endocardial radioactivity in the ischemic area (endocardial/epicardial radioactivity ratio falling from 1.17 to 0.76, P < 0.001) but not in the nonischemic area. Both nitroglycerin (0.4 mg) and propranolol (1 mg/kg) failed to cause a significant change in the ratio of circumflex to descendens radioactivity during ischemia. They did, however, cause a significant increase in the ratio of endocardial to epicardial radioactivity in both ischemic and nonischemic areas.

KEY WORDS: nitroglycerin, acute coronary artery occlusion, collateral flow

Radioactive microspheres have been used in experimental animals to estimate regional myocardial blood flow (1, 2). This technique has the advantage over previous methods in that multiple determinations can be made in the same animal, thereby allowing each to serve as its own control. In the present investigation the distribution of radioactive microspheres was determined during acute coronary artery occlusion in anesthetized dogs. These studies suggest that within an ischemic area of myocardium, there is a disproportionate subendocardial ischemia. During acute coronary artery occlusion nitroglycerin and propranolol were found to increase the ratio of endocardial to epicardial radioactivity.

Methods
The microsphere technique for studying regional myocardial blood flow is based on the principle that a nondiffusible indicator injected into the circulation distributes according to blood flow during its first transit (3). Microspheres are injected into the left atrium and are carried with the flow of blood to the systemic circulation and coronary arteries. Because of their size they lodge in precapillary vessels and do not recirculate (1, 2). Later, the hearts are removed and the radioactivity of selected areas determined, the amount of activity being a measure of the number of microspheres, which in turn is a reflection of blood flow to that area. Although large doses of microspheres can alter systemic hemodynamics, it has been shown that the amounts used in the present experiments do not result in significant alteration of heart rate, aortic pressure, cardiac output, coronary blood flow, or reactivity of the coronary vascular bed (2).

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were used in the present studies. They were obtained as 1 mc of nuclide in 10 ml of 10\% dextran to which one drop of Tween 80 was added to minimize clumping. The specific activities for the three isotopes were 13.8 mc/g for Ce, 63.5 mc/g for Cr, and 9.4 mc/g for Sr. The dose consisted of 100,000 to 500,000 microspheres per injection; they were given as a bolus in 0.1 to 0.2 ml, and the injection system was flushed with 2 to 3 ml of saline.

Twenty-eight mongrel dogs, weighing 15 to 25 kg, were anesthetized with intravenous sodium pentobarbital, 25 mg/kg, and given positive pressure respiration through a cuffed endotracheal tube. Polyethylene catheters were placed in the central aorta and jugular veins. A left thoracotomy was performed and a catheter placed in the left atrium through an atriotomy and a polyethylene snare placed around the left circumflex coronary artery. The electrocardiogram and arterial blood pressure were recorded. The first injection of microspheres was made into the left atrium during a control period. After 15 minutes acute ischemia was produced in the circumflex area by complete occlusion of the snare. Five seconds after the onset of the occlusion, a second injection of spheres labeled with a second nuclide was made into the left atrium. The occlusion was held for a total of 35 seconds, allowing the spheres to distribute during the period of ischemia. After 15 to 20 minutes one of three agents was given intravenously. Ten of the 28 dogs received propranolol 1 mg/kg, 11 received nitroglycerin 0.4 mg, and the remaining 7 received saline, serving as a control for the two drug groups. Five minutes after drug administration, a period of ischemia identical to the first was produced by occlusion of the snare, and five seconds after the onset of the occlusion, a third injection of spheres labeled with a third nuclide was made; the occlusion was again held for a total of 35 seconds. Although the 35-second occlusion may not have represented a steady state, the saline group was treated in exactly the same manner as the two drug groups and thereby provided an adequate control. In these experiments the distribution of the first nuclide corresponded to the control situation, the distribution of the second nuclide to acute ischemia, and the distribution of the third nuclide to acute ischemia with drug pretreatment. Ten minutes after the third injection, the dogs were sacrificed with a large intravenous dose of sodium pentobarbital, and the hearts were excised and washed free of blood. The epicardial fat and large epicardial vessels were cut away. The left ventricular free wall was removed and divided roughly into the areas supplied by the left circumflex (C) and left anterior descending (D) coronary arteries, each of which was then subdivided into inside (endocardial) and outside (epicardial) halves. Approximately 10 g samples from the center of each area were weighed, placed in plastic vials, and counted in a well counter at each of three different energy windows (75 to 175, 250 to 375, 400 to 600 keV). With the aid of an IBM computer, the counts for each isotope were separated by differential spectrometry (4) and the counts per gram of tissue for each nuclide in each area calculated. The results were expressed as the ratio of counts per gram of one area to another. Statistical significance of the results was determined using the Student t-test for sets of paired observations.

Results

The distribution of microspheres during the control period and during acute occlusion of the left circumflex coronary artery are shown in Table 1 for the entire group of 28 anesthetized dogs. During occlusion there was a significant fall in the ratio of circumflex/decescundens radioactivity and in endocardial/epicardial activity in the circumflex area. There was no significant change in the endocardial/epicardial ratio in the descendens area.

The effects of intravenous nitroglycerin and

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**TABLE 1**

<table>
<thead>
<tr>
<th>Effect of Acute Occlusion of Left Circumflex Artery in Twenty-eight Anesthetized Dogs*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/D</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Occlusion</td>
</tr>
</tbody>
</table>

*Key to abbreviations: C/D = ratio of counts per gram of circumflex to anterior descendens area myocardium; C endo/epi, D endo/epi = ratio of counts per gram of endocardial to epicardial halves in the area supplied by the left circumflex and anterior descending coronary arteries, respectively. Numbers are mean values with standard deviation. P values refer to difference between control and occlusion for each ratio; n.s. = not significant.
TABLE 2

Effect of Nitroglycerin and Propranolol During Acute Occlusion of Left Circumflex Coronary Artery*

<table>
<thead>
<tr>
<th></th>
<th>C/D Endo/epi</th>
<th>C Endo/epi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00</td>
<td>0.99</td>
</tr>
<tr>
<td>Occlusion</td>
<td>0.19f</td>
<td>1.00</td>
</tr>
<tr>
<td>Saline-Oclusion</td>
<td>0.22f</td>
<td>1.03</td>
</tr>
<tr>
<td>Control</td>
<td>0.98</td>
<td>1.06</td>
</tr>
<tr>
<td>Occlusion</td>
<td>0.19f</td>
<td>1.05</td>
</tr>
<tr>
<td>Nitroglycerin-Oclusion</td>
<td>0.22f</td>
<td>1.101</td>
</tr>
<tr>
<td>Control</td>
<td>0.98</td>
<td>1.06</td>
</tr>
<tr>
<td>Occlusion</td>
<td>0.23f</td>
<td>1.08</td>
</tr>
<tr>
<td>Propranolol-Oclusion</td>
<td>0.28f</td>
<td>1.081</td>
</tr>
</tbody>
</table>

*C,D, D endo/epi and C endo/epi ratios (see Table 1) are shown for control period, acute circumflex occlusion, and repeat occlusion with drug pretreatment. The animals are divided into three groups on the basis of the drug received (saline, nitroglycerin, or propranolol). Values given are the mean ratios, and n refers to the number of animals in each group.

fValue is statistically significantly different from control. fValue is significantly different from occlusion in each group (paired t-test, see Table 3).

Table 3

Statistical Analysis of Table 2*

<table>
<thead>
<tr>
<th></th>
<th>C/D</th>
<th>C Endo/epi</th>
<th>D Endo/epi</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>t</td>
<td>P &lt;</td>
<td>n</td>
</tr>
<tr>
<td>C vs. O</td>
<td>0.04</td>
<td>21.66</td>
<td>0.001</td>
</tr>
<tr>
<td>C vs. O + S</td>
<td>0.04</td>
<td>18.00</td>
<td>0.001</td>
</tr>
<tr>
<td>C vs. O + TNG</td>
<td>0.03</td>
<td>14.44</td>
<td>0.2</td>
</tr>
<tr>
<td>C vs. O + P</td>
<td>0.03</td>
<td>19.22</td>
<td>0.001</td>
</tr>
<tr>
<td>C vs. O</td>
<td>0.05</td>
<td>3.00</td>
<td>0.001</td>
</tr>
<tr>
<td>C vs. O + TNG</td>
<td>0.05</td>
<td>12.00</td>
<td>0.001</td>
</tr>
<tr>
<td>C vs. O + P</td>
<td>0.05</td>
<td>2.13</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Values for the standard error of the difference (se), t, and P are given for each of the comparisons in Table 2 using the paired t-test. C = microsphere distribution during control period; O = distribution during acute circumflex occlusion; O + S, O + TNG, O + P = distribution during second period of acute circumflex occlusion with drug pretreatment, where S = saline, TNG = nitroglycerin, and P = propranolol.

propranolol during acute myocardial ischemia are demonstrated in Table 2. The microsphere distribution during the control period, circumflex occlusion, and occlusion with drug are shown for each of the three groups: saline control (7 dogs), nitroglycerin (11 dogs), and propranolol (10 dogs). In the saline group there were no significant changes in any of the ratios between the first and second periods of ischemia. Nitroglycerin, given five minutes before the second ischemic period, caused a significant increase in the mean endocardial/epicardial ratio in both the descends and circumflex areas, while no significant change in the circumflex/descends activity was seen. Similarly, propranolol caused a significant rise in the endocardial/epicardial ratio in both ischemic and nonischemic areas but no significant change in the circumflex/descends ratio. Table 3 shows the standard error of the difference and values for t and P for each of the comparisons within drug groups in Table 2, using the paired t-test.
The systemic hemodynamic effects of nitroglycerin had disappeared by the time of sphere injection; the systolic blood pressure and heart rate were not significantly different from the values of 153 ± 14 mm Hg and 174 ± 26 beats/min in the control period. In the propranolol group the control systolic aortic pressure was 111 ± 23 mm Hg and heart rate 169 ± 24 beats/min; propranolol caused an 18% ± 10% decrease in heart rate (P < 0.001) but no significant change in systolic pressure. No significant correlation could be found between the decrease in heart rate and increase in the ratio of endocardial/epicardial radioactivity during ischemia after propranolol (Table 4).

**Discussion**

Microspheres have been used to measure blood flow to large regions of the heart, including the septum and right and left ventricular free wall (1, 2). The results with this technique agree with those found using 133Xe and 82Rb (5-9). The validity of the microsphere method as a measure of flow to the layers of the ventricular wall has, however, been questioned (1) since different endocardial-epicardial distributions are obtained with different sized microspheres (1, 2). This fact in itself does not invalidate the use of the small 15-μ spheres to estimate regional myocardial blood flow. The control endocardial/epicardial ratios of 1.04 to 1.17 which we have found in anesthetized dogs are similar to the results obtained with diffusible radioactive indicators. Almost all investigators have found endocardial activity to be equal to or slightly greater than epicardial (7, 9-14). An exception is the depot technique in which endocardial flow is found to be 25% less than epicardial (14). Objections have been raised to this method, however (15). At the present time there is no way to verify the accuracy of any available method of measuring regional blood flow to layers of left ventricular myocardium since no absolute standard exists. We feel, however, that 15-μ microspheres give a reasonably good estimate of flow distribution since our results agree with those found by previous techniques using diffusible indicators, both at rest and after various physiological interventions (2).

**Table 4**

<table>
<thead>
<tr>
<th>Dog</th>
<th>Heart rate</th>
<th>C endo/epi</th>
<th>C endo/epi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>O + P</td>
<td>%Δ</td>
<td>%Δ</td>
</tr>
<tr>
<td>1</td>
<td>186</td>
<td>138</td>
<td>17.0</td>
</tr>
<tr>
<td>2</td>
<td>189</td>
<td>162</td>
<td>10.0</td>
</tr>
<tr>
<td>3</td>
<td>185</td>
<td>138</td>
<td>17.0</td>
</tr>
<tr>
<td>4</td>
<td>216</td>
<td>138</td>
<td>26.1</td>
</tr>
<tr>
<td>5</td>
<td>186</td>
<td>144</td>
<td>22.6</td>
</tr>
<tr>
<td>6</td>
<td>180</td>
<td>138</td>
<td>20.3</td>
</tr>
<tr>
<td>7</td>
<td>144</td>
<td>120</td>
<td>12.5</td>
</tr>
<tr>
<td>8</td>
<td>138</td>
<td>126</td>
<td>8.7</td>
</tr>
<tr>
<td>9</td>
<td>144</td>
<td>144</td>
<td>17.2</td>
</tr>
</tbody>
</table>

*The heart rate (beats/min) and ratio of endocardial/epicardial radioactivity in the circumflex area (C endo/epi) are shown for nine dogs during acute circumflex occlusion (O) and repeat occlusion with propranolol pretreatment (O + P). The percentage change (%Δ) in each parameter between O and O + P is indicated. The correlation coefficient between these paired percentage changes was 0.27 (P < 0.5).
DISTRIBUTION OF RADIOACTIVE MICROSPHERES

within that area. The disproportionate reduction of spheres in the endocardium may be a consequence of a reduced perfusion pressure in the ischemic area. It has been shown by Moir and DeBra (12) and Griggs and Nakamura (13), using diffusible radioactive indicators, that partial coronary occlusion is accompanied by a disproportionate fall in endocardial flow. Our results are further supported by pathological data which show that myocardial necrosis is more extensive in the inner layers of the left ventricle following coronary artery occlusion (16, 17). Further, it has been shown that there is a significantly greater fall in ATP and phosphoryl-creatine and a rise in the lactate/pyruvate ratio in the inner compared with the outer layers of the myocardium during reduced coronary perfusion (18).

EFFECT OF ANTIANGINAL DRUGS ON MICROSPHERE DISTRIBUTION

No significant change in microsphere distribution between circumflex (ischemic) and descendens (nonischemic) areas of myocardium was seen after nitroglycerin or propranolol. Although it has been proposed that both nitroglycerin (19) and propranolol (20) may cause a shift in regional blood flow from nonischemic to ischemic areas, we were unable to demonstrate such a shift in microsphere distribution. However, it is probable that our acutely ischemic animals had only small native collaterals and that significant redistribution of flow after nitroglycerin was not possible because of the limitation of collateral vessel size.

Although no increase in the distribution of microspheres to the ischemic area was seen, both nitroglycerin and propranolol caused a significant increase in the ratio of endocardial to epicardial radioactivity. This increase occurred in both ischemic and nonischemic areas. Absolute levels of coronary blood flow were not measured in the present experiments, but previous work would suggest that flow was maintained in the ischemic area after both nitroglycerin and propranolol (19, 21-25). Five minutes after intravenous nitroglycerin, total coronary flow is unchanged or increased (21, 22), and retrograde flow to the ischemic area relative to the systemic blood pressure is also increased (19). After propranolol total coronary flow may be decreased (23, 24), but flow to the ischemic area is maintained (25). These data suggest that at least in the ischemic area, an increase in ratio of endocardial/epicardial radioactivity indicates an absolute increase in endocardial radioactivity.

The redistribution of microspheres observed in these experiments suggests a similar change in regional myocardial blood flow. Although it is difficult to exclude completely the possibility that nitroglycerin and propranolol may have caused some change in the distribution of microspheres without a concomitant change in blood flow, the fact that similar results have been obtained with other methods of measuring regional myocardial blood flow supports our hypothesis (12, 26). Experiments by Winbury with an oxygen electrode have suggested that a redistribution of blood flow from epicardium to endocardium occurs after nitroglycerin. He found that tissue pO2 falls in both the endocardium and epicardium 30 seconds after nitroglycerin but that at 4 to 12 minutes the epicardial pO2 returns to control while the endocardial pO2 rises above control values (26). In the case of propranolol, studies with 86Rb in the nonischemic heart (12) have suggested that a relative increase in left ventricular endocardial flow occurs after its administration. Previous studies with microspheres (2) have also shown that propranolol causes a relative increase in endocardial radioactivity in the absence of ischemia.

The redistribution of microspheres found during myocardial ischemia after nitroglycerin and propranolol may be due to the peripheral actions of these drugs. Previous microsphere studies in nonischemic animals during the peak hypotensive effect of nitroglycerin had shown no significant change in the ratio of endocardial to epicardial radioactivity (2). Since reduced perfusion pressure is usually associated with decreased endocardial/epicardial flow (12), this finding suggested that nitroglycerin had an effect on the transmural

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distribution of microspheres which was being masked by its hypotensive effects. Therefore, in the present experiments nitroglycerin was studied five minutes after intravenous injection, at a time when systolic blood pressure and heart rate had returned to control values. It is likely, however, that venodilation with resultant pooling of blood was still present at this time (27-29); under these circumstances a decrease in heart size and wall tension would be expected (28, 29). This might in turn lead to a reduction in the gradient of tension within the ventricular wall, normally highest in the inner layer (30), permitting more spheres to enter the endocardium.

The peripheral action of propranolol leads to a diminution in heart rate and increased diastolic time per minute. Since endocardial flow occurs mainly in diastole (9, 12, 30), this increased diastolic time might be expected to increase endocardial flow. We were, however, unable to show a significant correlation between increased endocardial radioactivity and decreased heart rate after propranolol (Table 4). In view of the low correlation coefficient, factors in addition to heart rate, such as changes in the gradient of wall tension, may also play a role.

A direct action on myocardial vessels might also explain the effects of both nitroglycerin and propranolol. Such a mechanism would necessarily involve a differential change in vascular resistance between endocardium and epicardium leading to a redistribution of blood flow. Nitroglycerin has been shown to cause a transient decrease in small vessel resistance lasting one to two minutes and a longer lasting diminution in large (conduc-tive) vessel resistance for seven to ten minutes (31, 32). It is likely that nitroglycerin acts on the large perforating vessels which supply the endocardium as it does on the major epicardial arteries. Their dilatation might be expected to lead to a relative increase in endocardial flow. Propranolol, by beta-adrenergic blockade, leads to a passive rise in coronary vascular resistance because of unopposed alpha-receptor activity. Although it has been shown that there is a decrease in alpha-adrenergic receptor activity from the major epicardial coronary arteries to the more distal branches (33), the distribution of adrenergic receptors between endocardium and epica-ridium is unknown. It is therefore unclear what importance the direct effect of propranolol on coronary vasculature has in the redistribution of microspheres found after this agent.

The present experiments suggest that during acute coronary artery occlusion there is a change in the distribution of flow within the ischemic area in that there is greater endocar-dial than epicardial ischemia. Both nitroglycerin and propranolol appear to prevent this disproportionate endocardial ischemia by causing a relative increase in endocardial flow without an increase to the ischemic area as a whole. Although the effectiveness of these agents during myocardial ischemia may be explained on the basis of a reduction in myocardial oxygen consumption, their effects on regional myocardial blood flow, as demonstrated in the present experiments, may also play a role in their beneficial action.

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