Effects of Coronary Collaterals on Regional Myocardial Function during Temporary Coronary Occlusion and Hypoxic Coronary Perfusion

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Summary

The effects of coronary collaterals on regional myocardial function during temporary ischemia and hypoxia were studied in 12 open-chest dogs. Using an ultrasonic dimension gauge, systolic segment shortening in the left anterior descending coronary artery (LAD) area was measured at 1 min after the following three experimental conditions: LAD occlusion and LAD hypoxic perfusion with nonoxygenated solutions at two different pressures (60 mmHg and 120 mmHg). Collateral function was assessed by both LAD diastolic retrograde pressure and the percentage of increase in left circumflex coronary flow at 1 min after LAD occlusion. Systolic segment shortening decreased less with hypoxic perfusion than with occlusion, however, this beneficial effect on regional contraction was greater at a perfusion pressure of 60 mmHg than at one of 120 mmHg. The magnitude of decrease of systolic shortening was variable among individual dogs but correlated linearly with each of the two collateral function indexes, not only during occlusion but also during hypoxic perfusion.

In conclusion, the preventive effect of hypoxic coronary perfusion on the early decline of regional myocardial function, in comparison to the changes seen during ischemia, may depend on coronary collaterals in addition to its washout effect on metabolites. In order to maintain myocardial function, perfusion pressure should be at an optimal level.
It is known that the early decline of cardiac function in myocardial ischemia is prevented by the perfusion of fluids with low oxygen content into the coronary artery, and this effect may be attributed to the washout by perfusing fluids of those metabolites which may cause deterioration of myocardial contraction and suppression of anaerobic energy production.1)-3) However, the effect of hypoxic coronary perfusion on regional contraction has not been evaluated quantitatively, and the relationship between the effects of hypoxic perfusion and coronary collaterals remains unclear.

Therefore, this study was performed in order to investigate the relationship between the effects of temporary hypoxic coronary perfusion and coronary collateral function, and also to investigate the effects of different hypoxic coronary perfusion pressures on regional contraction. We measured myocardial segment shortening in dogs with variable collateral functions during hypoxic coronary perfusion at two different perfusion pressures and compared them with that during coronary occlusion.

**Methods**

*Experimental preparation:* Studies were performed on 12 adult mongrel dogs weighing 9 to 11 kg. The dogs were anesthetized with 25 mg/kg of intravenous sodium pentobarbital, intubated, and supported with room air using a Harvard respirator (25 ml/kg). The heart was exposed through the 4th intercostal space and supported in the pericardial cradle. A 7F heparin-filled catheter was introduced into the aortic arch through the femoral artery for measurement of central aortic pressure. A polyethylene tube, whose internal diameter was 3 mm, was introduced into the aortic arch through the left carotid artery for coronary perfusion. The proximal portion of the left circumflex coronary artery (LC) was dissected free and a 2.0 or 2.5 mm flow probe attached to an electromagnetic flowmeter (Nihon Kohden, MFV-1200) was inserted. The left anterior descending coronary artery (LAD) was also dissected free at the proximal portion beyond the first diagonal branch, and after administration of heparin (5,000 units), it was ligated at that site, cannulated just distal to the ligature with a 19-gauge short cannula and perfused through the tube advanced from the left carotid artery. Initiation of this procedure interrupted perfusion for about 1–2 min, but regional wall motion was believed to have
recovered completely within 20 min. In the perfusion-line, a 3 mm extracorporeal flow probe attached to another flowmeter (Nihon Kohden, MFV-1200) and 2 three-way stopcocks were included. The stopcock near the cannula, connected to a pressure transducer (Nihon Kohden, MPU-0.5A), permitted continuous measurements of perfusion pressure or retrograde pressure during LAD occlusion. The other stopcock was used for LAD occlusion or perfusion from the reservoir which contained nonoxygenated lactated Ringer's solution. The distance from the latter stopcock to the tip of the inserted cannula was shorter than 10 cm and the dead space was less than 1 ml. LAD perfusion pressure through this tubing was about 10% less than aortic pressure, but peak reactive hyperemic flow, induced by 20 sec of occlusion of this tubing, was more than twofold that of the baseline flow. Therefore, the LAD seemed to be perfused sufficiently.4

A pair of 5-MHz ultrasonic crystals (2 mm in diameter, Schusler) were implanted subendocardially in the LAD area, approximately 1 cm apart and oriented in the direction of the circumferential fibers. The segmental myocardial length was measured with an ultrasonic dimension system (Schusler). It was ascertained by observation that the crystals were centered in the area where cyanosis or abnormal wall motion appeared during LAD occlusion.

An 18-gauge needle was inserted into the apex of the left ventricle and connected to a pressure transducer (Nihon Kohden, MPU-0.5A) via a heparin-filled short, stiff tube. Electrodes were attached for recording limb lead II of the electrocardiogram.

Experimental protocol:

In all 12 dogs various parameters were measured before and 1 min after the following three experimental conditions: (1) LAD perfusion-line occlusion; (2) LAD perfusion with nonoxygenated lactated Ringer's solution at a constant pressure of 60 mmHg; (3) LAD perfusion with the same solution at a constant pressure of 120 mmHg. The two perfusion pressures of 60 mmHg and 120 mmHg, which were controlled by the height of the reservoir, were used to investigate the effects of different hypoxic perfusion pressures on regional contraction. Unlike a hypoxic perfusion pressure of 60 mmHg, a perfusion pressure of 120 mmHg is so much higher than the aortic pressure that the collateral blood flow should be impeded considerably. In order to minimize the effect on hemodynamics and coronary vascular beds of perfusing the Ringer's solution at a rate of more than 20–40 ml/min, these procedures were performed in the order mentioned above. Between every two experimental conditions, the dogs were allowed to recover for at least 10 min.

The lactated Ringer's solution (Ohtsuka Pharmaceutical Co.) contained in mmol/liter: Na 130; K 4.0; Ca 1.5; Cl 109; lactate 28. Before perfusion,
the solution was neutralized to pH 7.5±0.3 with 1N NaOH and warmed to 38°C, its oxygen content was less than 0.5 Vol%. Because the solution contained lactate and no glucose, its osmotic pressure was isotonic to serum and the possibility of anaerobic energy production due to extrinsic glucose was excluded.

**Measurements:**

The electrocardiogram, central aortic pressure, LAD perfusion pressure, left ventricular pressure, LC blood flow, LAD perfusing flow and ultrasonic segment length gauge signal in the LAD area were recorded on an 8-channel pen recorder (Nihon Kohden, RM-6000) at 2 paper speeds, 1 and 100 mm/sec. One minute before and after each experimental condition, recordings were made at a paper speed of 100 mm/sec with respiration suspended transiently at end-expiration. During the higher speed recordings, phasic changes in coronary flow and left ventricular end-diastolic pressure with gain control were measured. For the purpose of timing cardiac events, end-diastole was defined as the peak of the R-wave of the electrocardiogram and end-systole was defined as the dicrotic notch in the central aortic pressure.

The values for end-diastolic segment length (EDL) were normalized to an initial EDL of 10 mm by dividing the measured EDL by the initial EDL and multiplying it by 10. Systolic segment shortening was calculated by the formula: (end-diastolic length - end-systolic length)/(end-diastolic length) × 100. To compare the decrease in systolic segment shortening among individual dogs, the percentage of change in systolic shortening from the baseline value was calculated in each experimental condition.5

LAD diastolic retrograde pressure following LAD occlusion6)-8) and the percentage of increase from baseline in LC flow at 1 min after LAD occlusion, which was proposed as the "donor coronary inflow method",9) were used for assessment of coronary collateral function in each dog.

**Statistics:**

All values are presented as mean±SE. The baseline values of the three experimental conditions were compared by a repeated measures analysis of variance.10) The paired data in each condition were analyzed by Student's t-test, and Tukey's test10) was used for paired comparison of the different effects among the three experimental conditions. Linear regression analysis was used to evaluate the correlation between the two collateral function indexes or between the percentage of change in systolic segmental shortening and either of the collateral function indexes. The level of statistical significance was p<0.05.
RESULTS

Hemodynamics, coronary flow and regional wall motion:

Representative records from 2 dogs are shown in Figs. 1 and 2.

Hemodynamics, coronary flow and regional wall motion in the three experimental conditions are shown in Table I. The baseline values of these measured variables were similar among the three conditions (not significant by a repeated measures analysis of variance).

Heart rate was reduced significantly during LAD hypoxic perfusions, but this decrease was less than 10% of the baseline value in every dog. Mean aortic pressure was reduced during LAD occlusion by 4±1 mmHg, while during hypoxic perfusion at 120 mmHg aortic pressure increased by 3±1 mmHg. Left ventricular end-diastolic pressure increased in every condition, but these increases did not differ significantly among the three conditions.

Following LAD occlusion, LC blood flow increased gradually and reached a plateau within about 30 sec, the magnitude of which varied among individual dogs as shown in Figs. 1 and 2. With hypoxic perfusion at 60 mmHg, LAD flow decreased transiently due to an abrupt decrease in perfusion pressure and then immediately increased to about twice the baseline flow.

Fig. 1. Representative record from a dog with poor collateral function. From top to bottom, electrocardiogram (ECG), aortic (AO) pressure, left anterior descendence (LAD), perfusion (PF) pressure, left ventricular (LV) pressure, LAD flow, left circumflex (LC) flow and segment length gauge signal from LAD area were recorded. Panels (from left to right) illustrate recordings in each experimental condition of LAD occlusion (OCC), hypoxic perfusion at 60 mmHg (PF60), and hypoxic perfusion at 120 mmHg (PF120). Recordings were made continuously at a slow speed, but fast recordings were taken 1 min before and after each experimental procedure. Note that the LAD segment showed severe dysfunction following OCC or PF120, but that during PF60 it showed hypokinesis. Additionally, low LAD retrograde pressure during LAD occlusion and a small increase in LC flow at 1 min after LAD occlusion were noted (left panel).
Fig. 2. Representative record taken from a dog with good collateral function. Each trace record and each panel are identical to Fig. 1. During OCC or PF120, the LAD segment showed mild hypokinesis, while during PF60 regional motion was hardly affected. Relatively high LAD retrograde pressure during LAD occlusion and an immediate increase in LC flow following LAD occlusion were noted (left panel). But with PF120, LC flow initially showed a dip due to high LAD perfusion pressure via collaterals and then, secondary to hypoxic vasodilatation in the LC area, LC flow increased.

Table I. Hemodynamics, Coronary Flows and Regional Wall Motion in Three Study Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>LAD occlusion before</th>
<th>LAD occlusion after</th>
<th>LAD hypoxic perfusion at 60 mmHg before</th>
<th>LAD hypoxic perfusion at 60 mmHg after</th>
<th>LAD hypoxic perfusion at 120 mmHg before</th>
<th>LAD hypoxic perfusion at 120 mmHg after</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDL (mm)</td>
<td>10</td>
<td>11.2±0.2*</td>
<td>9.9±0.1</td>
<td>10.5±0.1*</td>
<td>10.1±0.1</td>
<td>11.0±0.2*</td>
</tr>
<tr>
<td>% SS</td>
<td>19±1</td>
<td>-2±2*</td>
<td>19±1</td>
<td>9±3*</td>
<td>19±1</td>
<td>2±2*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>132±7</td>
<td>135±6</td>
<td>132±6*</td>
<td>134±6</td>
<td>131±7*</td>
<td></td>
</tr>
<tr>
<td>mAoP (mmHg)</td>
<td>84±3</td>
<td>80±3*</td>
<td>84±3</td>
<td>82±4</td>
<td>85±4</td>
<td>88±4*</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>4.7±0.4</td>
<td>6.2±0.6*</td>
<td>4.7±0.5</td>
<td>5.9±0.6*</td>
<td>4.8±0.4</td>
<td>6.8±0.6*</td>
</tr>
<tr>
<td>LAD flow (ml/min)</td>
<td>12±2</td>
<td>0</td>
<td>11±1</td>
<td>22±2*</td>
<td>12±1</td>
<td>44±2*</td>
</tr>
<tr>
<td>LC flow (ml/min)</td>
<td>18±2</td>
<td>22±2*</td>
<td>18±2</td>
<td>21±2*</td>
<td>19±2</td>
<td>26±2*</td>
</tr>
</tbody>
</table>

Values are mean±SE.
* p<0.05 vs baseline values. Paired comparisons among conditions were analyzed by Tukey’s test.

EDL=end-diastolic length; HR=heart rate; LAD=left anterior descending; LC=left circumflex; LVEDP=left ventricular end-diastolic pressure; mAoP=mean aortic pressure; % SS=percent systolic shortening.
Table II. Collateral Indexes and Systolic Segment Shortening in Each Experimental Dog

<table>
<thead>
<tr>
<th>No.</th>
<th>LAD retrograde pressure S/D (mmHg)</th>
<th>%JLC flow</th>
<th>LAD occlusion</th>
<th>LAD hypoxic perfusion at 60 mmHg</th>
<th>LAD hypoxic perfusion at 120 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>before</td>
<td>after</td>
<td>% change</td>
</tr>
<tr>
<td>1</td>
<td>40/16</td>
<td>39</td>
<td>20</td>
<td>10</td>
<td>-50</td>
</tr>
<tr>
<td>2</td>
<td>15/5</td>
<td>15</td>
<td>16</td>
<td>-6</td>
<td>-138</td>
</tr>
<tr>
<td>3</td>
<td>47/17</td>
<td>34</td>
<td>16</td>
<td>13</td>
<td>-19</td>
</tr>
<tr>
<td>4</td>
<td>24/12</td>
<td>33</td>
<td>23</td>
<td>1</td>
<td>-96</td>
</tr>
<tr>
<td>5</td>
<td>16/6</td>
<td>13</td>
<td>19</td>
<td>-5</td>
<td>-126</td>
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<td>6</td>
<td>18/9</td>
<td>20</td>
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<td>-4</td>
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<td>12</td>
<td>16</td>
<td>-14</td>
<td>-186</td>
</tr>
<tr>
<td>8</td>
<td>20/10</td>
<td>11</td>
<td>28</td>
<td>-1</td>
<td>-104</td>
</tr>
<tr>
<td>9</td>
<td>22/10</td>
<td>20</td>
<td>19</td>
<td>-3</td>
<td>-116</td>
</tr>
<tr>
<td>10</td>
<td>36/12</td>
<td>25</td>
<td>15</td>
<td>-8</td>
<td>-153</td>
</tr>
<tr>
<td>11</td>
<td>31/12</td>
<td>31</td>
<td>25</td>
<td>1</td>
<td>-96</td>
</tr>
<tr>
<td>12</td>
<td>17/4</td>
<td>14</td>
<td>14</td>
<td>-10</td>
<td>-171</td>
</tr>
</tbody>
</table>

LAD = left anterior descending; % change = percent change of systolic shortening from baseline values; %JLC flow = percent increase in the left circumflex inflow; S/D = systolic/diastolic.
value. LC flow also increased approximately as much as it did with occlusion. With perfusion at 120 mmHg, LAD flow increased as soon as the perfusion began and then reached a plateau whose level was about fourfold that of the baseline value. Simultaneously, LC flow increased by $7 \pm 1$ ml/min, which was significantly greater than that seen with occlusion or perfusion at 60 mmHg ($p<0.05$ by Tukey's test). LAD diastolic retrograde pressure during LAD occlusion and the percentage of increase in LC flow at 1 min after LAD occlusion in each dog, which were used as indexes of coronary collateral function as shown in Table II, correlated linearly with each other ($r=0.88$, $p<0.001$).

During LAD occlusion or hypoxic perfusions, EDL increased significantly and the magnitude of this elongation was significantly smaller with perfusion at 60 mmHg than with occlusion ($p<0.05$ by Tukey's test). Systolic segment shortening in the LAD area decreased markedly 1 min after LAD occlusion from $19\pm1\%$ to $-2\pm2\%$ ($p<0.001$). In contrast to occlusion, hypoxic perfusion at 60 mmHg reduced systolic shortening moderately from $19\pm1\%$ to $9\pm3\%$ ($p<0.01$). With perfusion at 120 mmHg, these values were from $19\pm1\%$ to $2\pm2\%$ ($p<0.001$). There was a significant difference in the magnitude of the decrease in systolic shortening between occlusion and perfusion at 60 mmHg ($p<0.01$ by Tukey's test), but the difference between

![Fig. 3](image-url)
occlusion and perfusion at 120 mmHg was not significant (Fig. 3).

**Relationship between regional myocardial function and collateral function:**

During LAD occlusion the percentage of decrease in systolic segment shortening correlated linearly with the coronary collateral function indexes, i.e., LAD diastolic retrograde pressure during LAD occlusion \( r = 0.88, p < 0.001 \) (Fig. 4) and the percentage of increase in LC flow at 1 min after LAD occlusion \( r = 0.75, p < 0.01 \) (Fig. 5).

Similar linear correlations were also found with hypoxic perfusions as

![Diagram](image_url)

**Fig. 4.** Relationship between left anterior descending (LAD) diastolic retrograde pressure following LAD occlusion and the percentage of change in systolic segment shortening from the baseline value in each experimental condition. Data from each dog following LAD occlusion (OCC, left panel), hypoxic perfusion at 60 mmHg (PF60, middle panel) and hypoxic perfusion at 120 mmHg (PF120, right panel) are denoted by ●, △ and □, respectively. Each linear correlation coefficient and its statistical level are shown in each panel.

![Diagram](image_url)

**Fig. 5.** Relation between the percentage of increase in left circumflex (LC) flow at 1 min after left anterior descending occlusion and the percentage of change in systolic segment shortening from the baseline value in each experimental condition. Symbols are identical to Fig. 4.
shown in Figs. 4 and 5. During hypoxic perfusion at 60 mmHg, the decrease in systolic shortening correlated both with LAD diastolic retrograde pressure during LAD occlusion (r=0.86, p<0.001) and with the percentage of increase in LC flow at 1 min after LAD occlusion (r=0.69, p<0.05). Additionally, with hypoxic perfusion at 120 mmHg there were correlations between the decrease of the systolic shortening and each of the two collateral function indexes, i.e., LAD diastolic retrograde pressure during LAD occlusion (r=0.79, p<0.01) and the percentage of increase in LC flow at 1 min after LAD occlusion (r=0.58, p<0.05).

DISCUSSION

The present study showed that systolic segment shortening in the LAD area decreased markedly during ischemia, while during hypoxia the decrease was attenuated as previously reported. This attenuation during hypoxia was more obvious with perfusion at 60 mmHg than with perfusion at 120 mmHg. Furthermore, it was shown that the percentage of decrease in systolic segment shortening correlated with the two indexes of coronary collateral function, not only during ischemia but also during hypoxic perfusions. Accordingly, though the decrease in systolic shortening during hypoxia was smaller than that seen during ischemia, the beneficial effects of hypoxic perfusion on regional function are considered to be dependent of perfusion pressure. In addition, it is suggested that regional function during hypoxia is affected by collateral function as it is during ischemia.

Previous studies reported that regional cardiac function provides more direct and precise assessment of the effects of myocardial ischemia or other interventions on myocardial performance than does total cardiac function. In addition, a close correlation between systolic segment shortening and regional blood flow has been well documented both in acute reduction in coronary blood flow and in temporary coronary occlusion when regional blood flow is derived from collaterals. Therefore, using an ultrasonic dimension gauge technique, we investigated quantitatively the different effects of temporary ischemia and hypoxia on regional function. We also examined whether the indexes used in this study to evaluate collateral function were proper or not by analyzing the relationship between these collateral indexes and the decrease in systolic shortening during coronary occlusion.

In order to evaluate collateral function precisely, regional blood flow during ischemia or hypoxia had to be measured. However, in this study, for simplicity and in order to reduce myocardial damage, we measured
LAD diastolic retrograde pressure during LAD occlusion and the percentage of increase in LC flow at 1 min after LAD occlusion. Although coronary retrograde pressure during its occlusion cannot be a very reliable index of collateral flow,\(^{18,19}\) it was often used to estimate collateral function\(^{6,7,20,21}\) and its diastolic pressure was thought to reflect collateral flow\(^8\) because extravascular compressive force is minimum during diastole. The percentage of increase in coronary flow following occlusion of another coronary artery was proposed by Cibulski et al\(^9\) as an index for assessing collateral flow. In this study the fact that these two indexes, i.e., LAD diastolic retrograde pressure during LAD occlusion and the percentage of increase in LC flow at 1 min after LAD occlusion, correlated linearly with each other and also with the percentage of decrease of systolic shortening during LAD occlusion indicates the appropriateness of these indexes for assessing collateral function. Nevertheless, the reason why the percentage of increase in LC flow did not correlate as well as the LAD diastolic retrograde pressure did may be explained by the fact that collateral flow was derived not only from the LC but also from other arteries, as well as the fact that the increased LC flow may partly be due to the compensatory increment of myocardial contraction in the LC area following LAD occlusion.

The effects of hypoxic perfusion pressure on collateral flow are surmised by changes in LAD and LC flow. Although it is also possible that perfusion with lactated Ringer’s solution can increase LAD flow due to its lower viscosity and hypoxic vasodilating effect, the markedly increased LAD flow seen during perfusion at 120 mmHg compared to that at 60 mmHg is most probably based on an elevation of perfusion pressure. Actually, at this elevated perfusion pressure we observed a pale area resulting from hypoxic perfusion extending to the area perfused by the LC. Accordingly, the LC area, also being subjected to hypoxic vasodilatation, might experience a significant increase in LC flow.

Concerning the different effects of ischemia and hypoxia on the early decline of cardiac function, two major explanations have been postulated. One is that the hypoxic perfusion causes washout of metabolites,\(^{1,5,22-24}\) such as hydrogen ion, lactate, phosphate and potassium, that seem to adversely affect myocardial contractions and suppress anaerobic energy production; the other explanation is attributed to mechanical force, called “the garden-hose effect”, which is the effect of perfusion flow or pressure for distending the myocardium.\(^{25}\) However, it is still controversial whether the washout effect promotes anaerobic energy production.\(^{25,26}\) Also, in this study regional function was only minimally improved by hypoxic perfusions compared to that with occlusion, in dogs with poor collateral circulation. On
the other hand, the garden-hose effect seems not to suffice as an explanation for our results because regional function was maintained better by perfusion at 60 mmHg than at 120 mmHg. Thus, we propose that the effects of hypoxic perfusion on regional function are also related to the degree of collateral function.

In conclusion, the prophylactic effect of hypoxic coronary perfusion on the early decline of regional myocardial function, in comparison to the decline in regional function seen during ischemia, may depend on coronary collateral function in addition to the effects of perfusion on the washout of metabolites. It is also concluded that, in order to maintain regional function, hypoxic perfusion pressure should be at an optimal level in order to not impede collateral flow.

REFERENCES

1. Petropoulos PC, Meijne NG: Cardiac function during perfusion of the circumflex coronary artery, with venous blood, low-molecular dextran, or Tyrode solution. Am Heart J 68: 370, 1964