Duration of Ischemia Is Vital for Collateral Development: Repeated Brief Coronary Artery Occlusions in Conscious Dogs

Masahiro Mohri, Hitonobu Tomoike, Mitsuru Noma, Takeshi Inoue, Katashi Hisano, and Motoomi Nakamura

The effects of two types of repeated transient coronary artery occlusions on collateral development were examined in chronically instrumented, conscious dogs. A 2-minute coronary occlusion at 32-minute intervals (group 1, n=11) or a 15-second occlusion at 4-minute intervals (group 2, n=7) were repeated day and night without interruption. In both groups, the total duration of coronary occlusions each day was the same (90 minutes). Before and after repetitive occlusions of either group, effects of transient 2-minute coronary occlusion on regional segment shortening in the ischemic area were examined to assess the functional state of the collateral vessels. In group 1, systolic segment shortening in the area rendered ischemic was reduced to $-97.8\pm17.7\%$ of the preocclusive control value during 2 minutes of coronary occlusion. After 125-478 repetitive occlusions (3-11 days), the degree of hypokinesia during the 2-minute occlusion was significantly improved to $-0.6\pm4.6\%$ of the preocclusive value ($p<0.001$ vs. before the repetition). In group 2, it remained unchanged even after 3,500-5,450 repetitive occlusions (11-16 days): $-111.8\pm8.2\%$ before and $-111.4\pm13.8\%$ after the repetition of 15-second occlusions (NS). The ratio of peripheral coronary arterial pressure to aortic pressure, measured by selective catheterization, was significantly higher in group 1 than in group 2 (64.4±5.3\% vs. 20.7±1.3\%, $p<0.001$). These findings suggest that myocardial ischemia of 2 minutes but not 15 seconds is vital to provide effective stimuli for angiogenesis.

Coronary collateral vessels are potential sources of the blood supply for myocardium jeopardized by severe stenosis and/or obstruction in the large epicardial coronary artery.1-2 Collateral circulation is angiographically evident only when the degree of arterial luminal narrowing exceeds 90% in patients with coronary artery disease.3 A similar phenomenon has been confirmed in animal experiments in which an ameroid constrictor was placed around the coronary artery and collateral development was anatomic and physiologically documented during chronic myocardial ischemia induced by gradual coronary obstruction.4-6

Angiographic studies in patients with vasospastic angina revealed a transient appearance of collateral channels only during coronary spasm, without significant coronary stenosis.7,8 This phenomenon was also observed in experimental studies.9,10 All these lines of evidence suggest that intermittent myocardial ischemia and/or pressure gradient between the intact and occluded coronary arteries can stimulate the development of coronary collateral vessels. However, the mechanisms of collateral development have not been elucidated, either experimentally or clinically.

Coronary collateral development is a process of active cellular proliferation.11,12 Recent studies revealed the important role of several angiogenic factors in initiating neovascularization in cases of tumor growth or wound healing.13 It is postulated that angiogenic factor(s) may be released from the myocardium during brief coronary occlusion. However, neither the precise characteristics of materials responsible for angiogenesis nor the stimulus for the
release have ever been rigorously evaluated. The present study was designed to determine the effective duration of coronary artery occlusion required to augment development of the coronary collaterals.

Materials and Methods

An Animal Model

Disease-free adult mongrel dogs of either sex, weighing 20–32 kg, were anesthetized with sodium pentobarbital (25 mg/kg i.v.) and were then ventilated with room air with a positive pressure respirator. Under sterile conditions, a left thoracotomy was performed in the fourth intercostal space, and an 8F polyvinyl chloride catheter was inserted into the aortic arch via the left internal thoracic artery to monitor systemic arterial pressure. After incising the pericardium, a high fidelity micromanometer (P-22, Konigsberg Instruments, Pasadena, California) and an 8F rigid polyvinyl catheter for calibration were inserted into the left ventricular cavity through a stab wound at the apex. The left circumflex coronary artery (LCx) was dissected free from its origin, and a pneumatic cuff occluder was placed around it. A 20-MHz pulsed Doppler flow probe (designed and prepared in our laboratory) or an electromagnetic flow probe (Nihon Kohden, Tokyo, Japan) was positioned just proximal to the occluder, so that no branch was present between them. Two pairs of 5-MHz piezoelectric crystals were implanted subendocardially into the left ventricular wall to measure the regional segment lengths, as described. Briefly, one pair of ultrasonic crystals was placed in the center of the area supplied by the LCx to provide a length of ischemic segment, and the other in the area supplied by the left anterior descending artery, as a control. At autopsy, all crystals were firmly embedded in the inner third of the left ventricular wall. Surface electrodes were sutured to the right atrial appendage, the right ventricular wall, and the center of the ischemic zone to monitor the epicardial electrocardiograms.

The pericardium was left open, and all wires and tubings were passed subcutaneously to the base of the neck and secured between the scapulae. The chest was closed, and the antibiotics, ampicillin and/or streptomycin, were given intramuscularly for 7 days after the operation.

Experimental Protocol

Repetition of coronary artery occlusions. All studies were carried out 10–14 days after the initial surgery. All dogs were active and fully recovered from the surgery; they were free from evidence of ill health and consumed the usual chow diet. The experimental room was dimly illuminated and kept free from noise or other activities. Every dog was well trained to lie quietly on its right side. After control recordings of electrocardiogram, left ventricular pressure (LVP), ischemic and nonischemic segment lengths, flow velocity or volume of the LCx, and aortic pressure (AoP), 2-minute abrupt coronary artery occlusion was performed by a cuff occluder to assess the functional significance of native collateral channels. Signals were continuously recorded until all variables returned to the preocclusive levels, on a pen recorder (NEC Sanei, Tokyo, Japan) at a paper speed of 50 mm/sec and on a magnetic tape (280-LT, TEAC, Tokyo, Japan) for later analysis.

Our telemetry system was designed to monitor a high fidelity LVP, two sets of segment lengths, and an electrocardiogram from an untethered animal at a remote place (Figure 1). Amplifiers for electrocardiogram, LVP, two-channel sonomicrometers, and FM-transmission were incorporated into a small cabinet (140 mm×90 mm×20 mm; approximately 100 g in weight), which could be easily carried by the dogs. By replacement of the six R14 (SUM-2) batteries once a day, the system could function continuously for several weeks.

After the initial challenge of a 2-minute coronary occlusion, the electronic equipment, including an occluder-driving miniature servomotor and an FM/FM telemetry transmitter, were carried by the dogs in a back pack. The dog was then put into a cage in the room used for the experiments. The dogs were randomly allotted to two groups for subsequent studies. An occluder was inflated and deflated by a miniature servomotor that was radiocontrolled by a preprogrammable microcomputer (Figure 1). Signals from LVP, two sets of regional segment length, and surface electrocardiogram were continuously monitored by an FM/FM telemetry system and were recorded on a pen-recorder at a paper speed of 5 mm/min and on a magnetic tape throughout the experiment.

GROUP 1. Group 1 consisted of 16 dogs in which 2-minute coronary artery occlusion was repeated every 32 minutes, continuously day and night. The measurement and recording of coronary blood flow were accomplished at least once a day. When regional shortening during a 2-minute coronary occlusion recovered to the preocclusive state, the repetition of 2-minute coronary artery occlusions was stopped.

GROUP 2. Group 2 consisted of 18 dogs in which 15-second coronary occlusion was repeated every 4 minutes in the same manner as group 1. This occlusion was continued at least 10 days (3,600 or more occlusions). A 2-minute coronary artery occlusion was then performed to assess functional state of the collateral vessel development.

In group 1, 2 minutes of coronary occlusion were performed 45 times per day, and in group 2, 15 seconds of coronary occlusion were performed 360 times per day. Accordingly, total duration in which the LCx was occluded was 90 min/day in both groups.

Coronary angiography and measurement of peripheral coronary artery pressure. After completion of the above protocol, dogs in both groups were
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FIGURE 1. Diagram of experimental system; see text for details. ECG, electrocardiogram; Seg, segment length; LVP, left ventricular pressure; DC-Amp, DC amplifier.

reanesthetized with intravenous sodium pentobarbital and ventilated with a positive pressure respirator. Selective coronary arteriography was performed with a preshaped green Kifa catheter in the left anterior oblique projection and was filmed at 48 frames/sec on 35 mm cinefilm (CSF746, Eastman Kodak, Rochester, New York) loaded on an Arriflex cinecamera (Arriflex, Munich, FRG). By manual injection of contrast medium (Urografin 76, Nihon Schering, Osaka, Japan), cineangiograms were taken before and at the end of the 2-minute coronary occlusion to assess the coronary stenosis around the cuff occluder and the degree of collateral development. Cinefilms were analyzed later on a viewing screen (Tagarno 35CX, Tagarno, Horsens, Denmark).

A rigid polyvinyl catheter (o.d. 0.9 mm, i.d. 0.5 mm) was inserted into a Kifa catheter and intro-
duced into the LCx under fluoroscopic guidance. After the tip of the catheter had been positioned beyond the implanted cuff occluder, peripheral coronary artery pressure (PCP) was monitored by attaching the catheter to a calibrated strain-gauge manometer (Statham P-23Ddb), associated with simultaneous measurement of AoP by another strain-gauge manometer set at the same level. After control recordings of LVP, AoP, PCP, and segment lengths, the LCx was abruptly occluded by inflation of the implanted occluder and was reperfused 2 minutes after the occlusion.

At the end of the experiment, the dog was given potassium chloride intravenously, the heart was excised, and the right and left coronary arteries were cannulated and gently flushed with saline. A barium-gelatin mixture was perfused into the coronary arteries at a pressure of 120 mm Hg. The heart was unrolled according to the Schlesinger's technique by separation of the right ventricular free wall and the interventricular septum from the left ventricle at the border. The unrolled heart was then fixed in a 20% buffered formalin solution. After fixation, stereoangiograms were taken.

Measurements

The miniature pressure gauge was calibrated in vivo by directly measuring LVP with a catheter attached to a calibrated strain-gauge manometer (Statham P23-Db, Gould Instruments, Cleveland, Ohio). Coronary blood flow of the LCx was measured with a flowmeter (545C-3, directional pulsed Doppler flowmeter) or with an electromagnetic flowmeter (Nihon Kohden). Measurement of AoP was taken by another calibrated strain-gauge manometer (Statham P23-Db). Transit time between a pair of ultrasonic crystals was converted by an ultrasonic dimension gauge (UDM-5C, MECC, Japan) to an analog voltage signal, which was proportional to instantaneous segment length. Calibration was performed by substitution of pulses with known duration. Mean values for AoP, coronary blood
FIGURE 2. Original tracings of left ventricular pressure (LVP), left ventricular dP/dt, segment lengths (Seg) in the left circumflex artery (LCx) and the left anterior descending artery (LAD) areas, phasic and mean LCx flow, and heart rate (HR) before (A), during (B; 2, 3, 4 = 2, 3, and 4 days after the beginning of repetitive occlusions), and after (C) repeated 2-minute coronary artery occlusions in group 1. Regional hypokinesia in the LCx area and hemodynamic derangement during 2-minute coronary occlusion were gradually improved as a function of time, along with a progressive diminution of reactive hyperemic response. On day 5, hemodynamic variables and segment shortening at 2 minutes of LCx occlusion returned to the preocclusive levels.

Flow velocity or volume, and PCP were obtained with a low-pass filter with a 2-second time constant. The first derivative of LVP (dP/dt) was obtained with an analog differentiator.

Measurement of segment lengths and calculation of regional segment performances were performed as described. Briefly, measurement of end-diastolic and end-systolic lengths (EDL and ESL, respectively) were taken at the initial rise of positive dP/dt and 20 msec before peak negative dP/dt, respectively. Segment systolic shortening was expressed as a percent fraction of EDL, which was termed as a percent segment shortening (%SS = 100 × (EDL − ESL)/EDL).

The degrees of the coronary stenosis around the implanted cuff occluder and of collaterals were assessed in vivo and postmortem by coronary angiograms, as reported. All data are presented as mean ± SD (standard deviation). Data were analyzed by Student's t test for paired data in hemodynamics and regional segment performances. Comparisons of mean values between groups were made by analysis of variance. The level of significance was p < 0.05.

Results

Five of 16 dogs in group 1 and five of 18 dogs in group 2 died of ventricular fibrillation during the procedure of repeated coronary occlusions. In another five dogs in group 2, significant fixed coronary artery stenosis, ranging from 90% to 99% luminal reduction assessed by selective coronary angiography, was noted at the site of the occluder, after 700–4,100 (2,500 ± 1,440) occlusions. All of these five dogs had developed collaterals originating from the left anterior descending artery to the stenosed LCx, and in two dogs, additional collaterals came from the right coronary artery. These five dogs were excluded from the following analysis. In one other dog in group 2, regional percent shortening at the first 2-minute occlusion of the LCx reduced by 32% from the preocclusive control value along with mild hemodynamic changes, thereby suggesting the presence of well-functioning...
Hemodynamic Responses and Regional Wall Motion During 2-Minute Coronary Occlusion Before Repeated Occlusions

Typical tracings are shown in Figure 2 and 3. Table 1 summarizes changes in heart rate (HR), LVP, left ventricular end-diastolic pressure (LVEDP), mean AoP, and peak positive and negative dP/dt. Changes in EDL and %SS in the control and ischemic regions before and during a 2-minute coronary occlusion are summarized in Table 2. Before repetitive coronary occlusions, an abrupt LCx occlusion caused similar changes in hemodynamics and in regional wall motion in the ischemic area between the two groups. Active systolic shortening was markedly depressed or replaced by paradoxical systolic lengthening in the area of ischemia, similarly in both groups 1 and 2. Quantitatively, %SS was decreased by \(-97.8\%\) from 19.7±6.0% to 1.1±2.9% in group 1, and by \(-111.8\%\) from 18.1±6.9% to \(-2.0±1.6\%\) in group 2 (not significantly different). In both groups, a prominent reactive hyperemic response was observed following release of occlusion of the LCx. These results suggested that the functional level of native collaterals was comparable in the two groups.

Hemodynamic Responses and Regional Wall Motion During 2-Minute Coronary Occlusion After Repetitive Coronary Occlusions

As shown in Figure 2, regional wall motion abnormality during 2 minutes of coronary occlusion was attenuated progressively with continuation of repetitive coronary occlusions in dogs of group 1. Hemodynamic deteriorations during the occlusion and reactive hyperemic response following release of the occlusion was also gradually attenuated. Hemodynamic variables at rest, including HR, LVP, LVEDP, mean AoP, and positive and negative dP/dt, and resting EDL and %SS in both control and ischemic regions were not significantly different compared with findings obtained before the repetitive occlusions (Tables 1 and 2). At the end of the repeated occlusions, HR, LVP, mean AoP, and LVEDP did not change significantly at 2 minutes of transient coronary occlusion (Table 1). Positive and
TABLE 1. Changes in Hemodynamics Before and at the End of 2-Minute Coronary Occlusion Before and After Repeated Coronary Occlusions

<table>
<thead>
<tr>
<th></th>
<th>Before RCO</th>
<th>After RCO</th>
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<tbody>
<tr>
<td></td>
<td>Group 1 (n=11)</td>
<td>Group 2 (n=7)</td>
</tr>
<tr>
<td></td>
<td>cont</td>
<td>2-min</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>90±11</td>
<td>123±14*</td>
</tr>
<tr>
<td>LVP (mm Hg)</td>
<td>111±15</td>
<td>103±11*</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>4.6±2.1</td>
<td>11.2±2.6</td>
</tr>
<tr>
<td>mAoP (mm Hg)</td>
<td>96±12</td>
<td>88±8*</td>
</tr>
<tr>
<td>(+)dP/dt (mm Hg/sec)</td>
<td>3,218±411</td>
<td>2,910±366*</td>
</tr>
<tr>
<td>(-)dP/dt (mm Hg/sec)</td>
<td>2,591±481</td>
<td>2,236±404*</td>
</tr>
</tbody>
</table>

Values are mean±SD. RCO, repeated coronary occlusions; HR, heart rate; LVP, peak left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; mAoP, mean aortic pressure; (+)dP/dt, peak positive left ventricular dP/dt; (-)dP/dt, peak negative left ventricular dP/dt; cont, preocclusive control; 2-min, at 2 minutes of coronary occlusion.

* p<0.01.
† p<0.05 (cont vs. 2-min).

negative dP/dt decreased transiently following coronary occlusion but gradually returned to control levels before release of occlusion. Regional segment shortening in the LCx area transiently decreased following the occlusion, but recovered to the preocclusive levels at 2 minutes of coronary occlusion (20.7±5.7% vs. 20.7±6.3%, NS). Changes in %SS during 2 minutes of coronary occlusion before and after the repetitive occlusions in all 11 dogs of group 1 are summarized in Figure 4. Namely, 125-478 (265±106) coronary occlusions of 2 minutes (3-11 days) were needed for the development of collateral circulation adequate to maintain resting regional systolic shortening during abrupt coronary artery occlusion.

In seven dogs of group 2, 3,500-5,450 coronary occlusions (4,400±630) were successfully repeated every 4 minutes for 11-16 days, continuously day and night (Figure 3). Resting hemodynamic variables and EDL and %SS in the control and ischemic areas were not significantly different compared with findings before the repetitive occlusions (Tables 1 and 2). After the completion of repetitive coronary occlusions, 2-minute coronary occlusion was again

TABLE 2. Changes in End-Diastolic Segment Length and Regional Fractional Shortening in Ischemic and Control Regions Before and at the End of 2-Minute Coronary Occlusion Before and After Repeated Coronary Occlusions

<table>
<thead>
<tr>
<th></th>
<th>Ischemic segment</th>
<th>Control segment</th>
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<tbody>
<tr>
<td></td>
<td>EDL (mm)</td>
<td>%SS (%)</td>
</tr>
<tr>
<td>Before RCO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1 (n=11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cont</td>
<td>13.4±2.3</td>
<td>19.7±6.0</td>
</tr>
<tr>
<td>2-min</td>
<td>14.2±2.4*</td>
<td>1.1±2.9*</td>
</tr>
<tr>
<td>Group 2 (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cont</td>
<td>12.7±3.2</td>
<td>18.1±6.9</td>
</tr>
<tr>
<td>2-min</td>
<td>13.9±3.3*</td>
<td>-2.0±1.6*</td>
</tr>
<tr>
<td>After RCO</td>
<td></td>
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</tr>
<tr>
<td>Group 1 (n=11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cont</td>
<td>14.0±2.6</td>
<td>20.7±5.7</td>
</tr>
<tr>
<td>2-min</td>
<td>14.0±2.7</td>
<td>20.7±6.3</td>
</tr>
<tr>
<td>Group 2 (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cont</td>
<td>12.5±3.7</td>
<td>16.2±4.6</td>
</tr>
<tr>
<td>2-min</td>
<td>13.5±4.0*</td>
<td>-1.8±2.7*</td>
</tr>
</tbody>
</table>

Values are means±SD. RCO, repeated coronary occlusions; EDL, end-diastolic length; %SS, percent segment shortening; cont, preocclusive control; 2-min, at 2 minutes of coronary occlusion.

* p<0.01.
† p<0.05 (cont vs. 2-min).
performed to assess the functional state of collaterals. Abrupt occlusion of the LCx caused hemodynamic declines and ischemic regional wall motion abnormality comparable to those observed before repetitive coronary occlusions (Figure 3, Tables 1 and 2); during 2 minutes of coronary occlusion, %SS was significantly decreased by -111.4±13.8% from 16.2±4.6% to -1.8±2.7%. Figure 4 shows changes in %SS during the 2-minute coronary occlusion before and after repetitive 15-second coronary occlusions performed in seven dogs of group 2. Regional myocardial dysfunction, measured as a percent reduction in the ischemic %SS during abrupt 2-minute coronary artery occlusion was unchanged after repeating 15-second coronary artery occlusions in all dogs of group 2.

Angiographic and Hemodynamic Assessment of Collateral Function

Before occlusion of the LCx, there was no significant coronary stenosis along the LCx in either group. With occlusion of the LCx by inflating the implanted cuff occluder, the epicardial portion of the LCx was immediately filled with collateral flow from the left anterior descending artery in group 1, whereas no branch of the LCx was filled after occlusion of the LCx in group 2. These differences in collateral filling of the LCx between two groups were also confirmed on the postmortem coronary angiograms.

Measurement of PCP was successful in five of 11 dogs in group 1 and in four of seven dogs in group 2. Before occlusion, PCP was 94.4±2.4% and 93.5±5.0% of AoP in group 1 and group 2, respectively, with no difference between the groups. After complete occlusion of the LCx by inflation of the implanted cuff occluder, PCP fell in both groups but was significantly higher in group 1 than in group 2 (69±12 mm Hg vs. 23±4 mm Hg, p<0.001). The ratio of PCP to aortic pressure was 64.4±5.3% in group 1 and 20.7±1.3% in group 2 (p<0.001).

Discussion

A great deal of knowledge concerning the pathophysiology of coronary collateral development has been attained through studies on animals instrumented with an ameroid constrictor, which causes gradual occlusion of the major coronary artery. Although such a gradual coronary occlusion stimulates collateral development, the rate of narrowing or the time required for complete occlusion of coronary artery varies with the species and/or animals and is difficult to control. To quantitatively assess the relation between the amount of stimulus devoted for collateral development and the level of collateral function, transient brief coronary artery occlusion has to be continuously repeated day and night, without interruption. For this purpose, we designed a radiocontrol system to regulate the implanted coronary occluder. Using this system, we defined the rate of collateralization as integrated numbers of abrupt coronary occlusion and compared the effects of repeated occlusions.

The level of collateral function developed was assessed by the regional systolic shortening during transient coronary occlusion in the ischemic area. The level of regional systolic fractional shortening is assumed to represent the level of collateral development, because regional function of the myocardium at jeopardy depends entirely on blood flow through collateral channels and the degree of hypokinesis along the endocardial segment in the center of ischemic area has been proved to correlate well with the absolute amount of endocardial blood flow, as measured with radioactive tracer microspheres both in open-chest dogs during coronary hyperperfusion and in conscious dogs under conditions of acute or chronic stenosis. A tracer microsphere technique has been accepted as the standard in determining collateral blood flow. However, its measurement is a relatively discrete one and the number of trials is limited. Determination of peripheral coronary pressure or retrograde flow has limited application and

![Figure 4. Changes in the degree of regional dysfunction induced by a 2-minute occlusion of the left circumflex coronary artery before and after repeated coronary occlusions (COs) in 11 dogs of group 1 (panel A) and in seven dogs of group 2 (panel B). Segment shortening during 2 minutes of occlusion is presented as a percent reduction (%R) from a preocclusive level. A: With 125-478 coronary occlusions (3-11 days), %R improved from -97.8±17.7% to -0.6±4.6% (p<0.001). B: Average percent reduction in ischemic regional segment shortening before and after 3,500-5,450 coronary occlusions (11-16 days) was -111.8±8.2% and -111.4±13.8%, respectively (NS).]
usefulness because of the requirement for intensive chronic catheterization or thoracotomy. Accordingly, assessment of regional systolic shortening during transient coronary occlusion in the collateral-dependent area is practical in reproducibility, repeatability, and facility in determining the functional state of developing collaterals in conscious animals.

The functional capacity of coronary collateral vessels can be enhanced with repetition of 2-minute coronary occlusions every 30 or 60 minutes, as reported from our laboratory and by other investigators. Similar observations were confirmed clinically for patients in whom repeated coronary artery spasms augmented collateral development without significant organic coronary stenosis or obstruction. Under these situations, myocardial ischemia, pressure difference across the native collaterals, or both may play important roles in initiating the collateral development. However, little is known of the duration of ischemia as an effective stimulus for collateral development. To clarify the physiological significance of 2 minutes of coronary occlusion with regard to the collateral development, effects of repeated 15 seconds of coronary occlusion at an interval of 4 minutes were compared in the present study. Although the total occlusion time per day was equally 90 minutes in both groups, the level of myocardial ischemia induced by each occlusion was much severer in group 1 than in group 2. Two minutes of coronary occlusion was accompanied by a full bulge in regional wall motion during coronary occlusion, whereas the level of regional hypokinesia observed after 15 seconds of coronary occlusion was about 40% of the full bulge observed during 2 minutes of coronary occlusion and recovery after reperfusion was rapid. However, the peak level of reactive hyperemia after 15 seconds of occlusion was similar to that seen with the 2-minute coronary occlusion.

Our observations clearly show that repetition of a 2-minute coronary occlusion at 32-minute intervals augments collateral function, with resultant attenuation of regional myocardial dysfunction. In contrast, a 15-second coronary occlusion failed to stimulate collateral development, despite the fact that the repetition was applied for longer periods. The degree of collateral development was also determined at the end of the study by the in vivo measurement of peripheral coronary pressure and selective coronary cineangiography. In dogs in group 1, peripheral coronary pressure was 69 mm Hg, 64% of aortic pressure, a level sufficient to maintain regional and global left ventricular function at rest. Our data coincide well with earlier work on the relation between coronary pressure and regional myocardial function or regional myocardial blood flow. Schaper demonstrated that the diastolic PCP exceeded 70% of the diastolic aortic pressure approximately 8 weeks after implantation of an ameroid constrictor. Since the time devoted for collateral development in the present study was less than 2 weeks; the collaterals developed in the present study were likely to be less mature than those developed by ameroid constrictor. The second possibility was the condition of forward coronary flow. Comparison of the complete cessation of forward coronary blood flow after occlusion by an ameroid ring showed that the coronary artery was patent during reperfusion intervals in the present animal model. Thus, blood flow through collateral channels may be absent or extremely small, and such a pause of flow signal as a stimulus may retard the development of collateral channels compared with findings when an ameroid is used.

Many investigators have attempted to elucidate the mechanisms of collateral development, but precise mechanisms remain in debate. Schaper noted a significant role of tangential wall stress acting on the collateral vessels as determinant factor for collateral development. Scheel and coworkers suggested that hypoxic stimulus may be the initiating step in the process of collateral growth and mechanical force may work to elicit subsequent structural changes of the vessel. Hollenberg and colleagues suggested the presence of unknown diffusible substance(s) that mediate angiogenesis during collateral development. Their investigations were confirmed to the renal circulation. Little is known of the pathophysiology of collateral development along with repeated brief coronary occlusions and resultant myocardial ischemia. In ameroid models, the coronary artery is subject to gradual narrowing, and eventually, complete occlusion and chronic myocardial ischemia is applied to the jeopardized myocardium. In contrast, in our repetitive occlusion model, the ischemic stimulus for collateral development is applied only during brief periods of coronary occlusion, and there is no myocardial ischemia or pressure gradient between major coronary arteries during the reperfusion periods. The finding that brief coronary occlusion augments functional capacity of collateral vessels implies that a 2-minute coronary occlusion is potentially effective as a stimulus for collateral development and that the stimulant effect accumulates along with the repetition.

Olsson and Gregg demonstrated that 5–7 seconds of coronary occlusion elicits the maximal dilation of the resistance vessels in the conscious dog. Olsson also showed that 15 seconds of coronary occlusion increased intramyocardial adenosine by a factor of 5. Thus, anaerobic substances, such as adenosine, may be sufficiently produced following 15 seconds of coronary occlusion. However, such vasodilator substances may not be of primary importance in directly initiating the collateral development in our model.

It also seems likely that cumulative periods of coronary artery occlusions do not play an important role, because repetition of a 15-second coronary occlusion for much longer integrated time than those of the 2-minute occlusion group did not stimulate collateral development. Accordingly, we assume that there is a qualitatively critical differ-
ence between these two types of brief coronary occlusions of 15 seconds and 2 minutes in capacity of stimulating the collateral function and that there is a threshold of ischemic stimulus for developing coronary collateralization.

Collateral vessel development is tightly coupled to the process of active cellular proliferation, as evidenced by titrated thymidine incorporation into endothelial, medial, adventitial, and myocardial mesenchymal cells.11,12 Why coronary collateralization was enhanced with repeated 2-minute coronary occlusions but not with 15-second ones is not readily apparent. Several studies have shown the important role of increased velocity of blood flow in initiating endothelial proliferation, possibly due to increased shear stress acting on the endothelial cells.35-38 In addition, stretching of the collateral channel by vasodilation and/or increased velocity may physically separate the endothelial cells, and the internal elastic lamina of the vessel would become fragmented, thus making way for cellular migration and proliferation.39 However, experimental studies10,40 and clinical observation41 suggested that collateral channels do not fully open within 30 seconds after abrupt coronary occlusion. Thus, it is possible that 15 seconds of coronary occlusion failed to open the preexisting collateral channels and thus initiate the process of angiogenesis.

Secondly, tissue ischemia is known to produce angiogenic factors. Galloway et al reported that an endothelial mitogen was released from the ischemic rabbit heart.42 Yang et al found that myocardial ischemia increased the levels and bioavailability of basic fibroblast growth factor.43 Kumar et al identified a low-molecular-weight angiogenic factor from human infarcted myocardial tissue.44 Similar results have been obtained from work on the renal circulation.29,31 Although the source of these ischemia-related angiogenic factors remains a matter of speculation, it seems plausible that a certain period of myocardial ischemia may be required to directly modulate synthesis and release of angiogenic factors.

In summary, the present study has added support to the proposal that repetitive brief coronary artery occlusion leads to development of collaterals. We also confirmed that coronary occlusion and/or myocardial ischemia of longer than 15 seconds is crucial in providing effective stimuli for angiogenesis. The substance(s) responsible for the development of coronary collateral vessels is the subject of ongoing studies.

Acknowledgments
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