Sensitization of Reperfused Myocardium to Subsequent Coronary Flow Reductions
An Extension of the Concept of Myocardial Stunning

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Robert A. Kieso, MS, and Richard E. Kerber, MD

The purpose of the present study was to evaluate the response of briefly ischemic and reperfused myocardium to subsequent moderate reductions of coronary arterial flow. In mongrel dogs, a carotid to left anterior descending coronary shunt was constricted to produce moderate coronary flow reductions (50–60% of control) and to thereby reduce regional systolic thickening (measured by echocardiography or sonomicrometry). First, we demonstrated an abnormal response of reperfused myocardium to subsequent flow reductions. We performed two episodes of coronary stentosis, with an intervening 5-minute complete coronary shunt occlusion followed by 30 minutes of reperfusion. In a control group, the same two stent stenoses were done, but no intervening shunt occlusion was performed. In the control dogs, repeated coronary stent stenosis that produced equivalent perfusion reductions also produced equivalent declines in regional wall thickening. In contrast, in the intervention group (animals undergoing the intervening occlusion-reperfusion sequence between two stent stenoses), the second coronary stent stenosis produced an exaggerated decline in regional systolic thickening, even though the decline in myocardial perfusion was similar to the first stenosis. Second, we sought to demonstrate the mechanism of the exaggerated decline of the reperfused myocardium to subsequent moderate flow reductions. Again, two groups of animals were studied. Each group underwent two episodes of coronary stent stenosis with an intervening sequence of 5 minutes of complete shunt occlusion and 30 minutes of reperfusion. In addition, one of the groups received an infusion of the oxygen free radical scavengers superoxide dismutase and catalase during the occlusion-reperfusion sequence. In the superoxide dismutase and catalase–treated animals, the decline in regional systolic function during the postreperfusion stent stenosis was similar to the preoclusion stenosis. Thus, oxygen free radical scavengers blocked the exaggerated contraction decline in response to the postreperfusion flow reduction. We conclude that briefly ischemic and reperfused myocardium displays an exaggerated response to subsequent coronary arterial flow reductions and that this response is a subtle manifestation of posts ischemic ventricular dyskinesis, or “stunning.” The mechanism is probably oxygen free radical toxicity. (Circulation 1988;78:717–728)

The extent of functional recovery after transient myocardial ischemia is strongly influenced by the duration and severity of the ischemia and the reestablishment of coronary perfusion.1–15 In experimental studies with sensitive techniques (sonomicrometry), single ischemic episodes of 15 minutes are responsible for prolonged regional dysfunction, but permanent injury after such occlusions does not occur.2–5 Such posts ischemic myocardial dysfunction without infarction has been termed “stunning.”15 After only 5 minutes of ischemia, the extent of recovery of systolic contraction has been variably reported; some investigators have found complete and rapid recovery,7–10 while others have shown residual impairment of function.4–6

Our hypothesis was that after a brief (5-minute) episode of myocardial ischemia, reperfused myocardium is, in fact, abnormal or stunned, but this
may be subtle. We proposed that these subtle abnormalities could be demonstrated by subjecting the reperfused myocardium to a subsequent moderate perfusion reduction. We suggested that this moderate perfusion reduction would result in more severe impairment of regional function than would occur when normal myocardium not previously subjected to a sequence of coronary occlusion-reperfusion was also subjected to moderate perfusion reductions. This exaggerated response is demonstrated in the present study and is referred to as "sensitization" of reperfused myocardium to subsequent moderate flow reductions—an extension of the concept of myocardial stunning. Furthermore, we show that the mechanism of this phenomenon is probably oxygen free radical toxicity because it can be blocked by administration of oxygen free radical scavengers during the occlusion-reperfusion sequence.

Materials and Methods

Experimental Preparation

Mongrel dogs weighing 16–24 kg were studied. All animals were subjected to graded coronary flow reductions. In group A (control) animals, two episodes of shunt stenoses were performed, but no other intervention was done. In groups B, C, and D, we subjected the animals to 5 minutes of complete coronary shunt occlusion between the two episodes of coronary shunt stenosis. Group D animals received the oxygen free radical scavengers superoxide dismutase and catalase during the period of complete shunt occlusion and reperfusion.

Anesthesia was induced with fentanyl-droperidol (0.13 ml/kg i.v.) followed by pentobarbital (20 mg/kg i.v.). Autonomic blockade was obtained by administering propranolol (1 mg/kg i.v.) and performing bilateral cervical vagotomy in an attempt to minimize arterial pressure fluctuations. Propranolol and vagotomy were omitted in groups C and D. Blood gases (four samples from each study) were kept in a physiological range by manipulating tidal volume, respiratory rate, and inspiratory oxygen content. A left thoracotomy was performed. The heart was elevated in a pericardial cradle. Complete ativoventricular block was obtained by injection of 0.3 ml 3.7% formaldehyde into the ativoventricular node. A constant heart rate at 90 beats/min was achieved by epicardial pacing with left ventricular apical electrodes connected to a Grass S9 stimulator. Left ventricular pressures were measured with a micromanometer-tipped Millar catheter (Houston, Texas) introduced retrograde. Left ventricular dP/dt was obtained by a differentiating amplifier with a linear response up to 60 Hz and a known analog display delay of 55 msec. In groups C and D, a fluid-filled 8F catheter was used to obtain left ventricular pressures. Ascending aortic pressure in all groups was obtained from a fluid-filled 8F catheter. Electrocardiogram, pressure, and dP/dt were recorded on a Beckman strip-chart recorder at 200 mm/sec.

After administration of heparin (500 units/kg) and acetylsalicylic acid (100 mg), the left anterior descending coronary and carotid arteries were cannulated and connected via a polyethylene tubing, thereby establishing a carotid-coronary shunt. The proximal left anterior descending coronary artery was ligated. The peripheral coronary pressure was measured at the intracoronary tip of the left anterior descending coronary artery cannula by a fluid-filled catheter connected to a 23 Pb Statham transducer (Cleveland, Ohio). The gradient between aortic and peripheral coronary arterial pressure during baseline (no coronary shunt stenosis) conditions never exceeded 6 mm Hg. A specially designed metallic cannula (diameter, 2.5 mm) interposed in the shunt included a pulsed-wave Doppler crystal (20 MHz) incorporated in the cannula at a fixed angle in relation to the flow of blood. This crystal was connected to a pulsed-wave Doppler unit specially designed, which provided mean and phasic velocity analog signals (Figure 1). Because the diameter of the shunt tubing was fixed as was the incident angle of a Doppler ultrasound beam, the Doppler velocimeter displayed a linear response to reductions of blood flow through the shunt. A mechanical occluder also incorporated in the shunt allowed stable and reproducible shunt stenosis or occlusion to be achieved. We used the Doppler recordings to estimate the degree of graded coronary flow reduction occurring during the coronary shunt stenosis to achieve equal flow reductions before and after the intervention of complete coronary occlusion. The peripheral coronary arterial pressures were also monitored and matched to achieve equivalent degrees of coronary shunt stenosis.

To measure myocardial perfusion, radiolabeled microspheres (diameter, 15 μm; 141Ce, 85Sr, 46Sc, 153GD, 113Sn, 95Nb, 20 μCi/injection; New England Nuclear, Boston, Massachussets) were injected into the left atrium while blood was being withdrawn simultaneously from two peripheral arterial cannulae.

In groups A and B, a specially developed 5-MHz M-mode echocardiographic transducer (diameter, 5 mm) was applied to the epicardium at the center of the left anterior descending artery perfusion area and fixed in place by a suction cup with a negative pressure of 3 mm Hg. This atraumatic ultrasonic device has been previously described in detail. It allowed stable recordings of instantaneous left ventricular wall thickness during each experiment. The echocardiographic, electrocardiographic, and left ventricular dP/dt signals were displayed on a strip chart recorder (Model 1602-B, Smith-Kline Instruments).

In groups C and D, we used another ultrasound technique, sonomicrometry, instead of the suction echo transducer. Two pairs of piezoelectric crystals were inserted into the left anterior descending cor-
Experimental Protocol

Recordings were obtained in the four groups of animals during the following experimental conditions: 1) preocclusion control (C1); 2) preocclusion graded coronary shunt constriction or stenosis (S1) to produce coronary flow reduction of moderate degree. This shunt constriction or stenosis was monitored with the Doppler flow velocimeter in the carotid-coronary shunt and the coronary pressure distal to the shunt. Flow reductions were achieved by constricting the carotid-coronary shunt 10 cm proximal to the Doppler velocimeter. A stable degree of flow reduction and hemodynamic response was achieved for at least 2 minutes before recordings were made. The shunt stenosis was released after recordings were completed. In groups A and B, this maneuver was performed twice. The total time of each individual coronary shunt stenosis never exceeded 4 minutes. 3) In control animals (group A), we allowed 30 minutes of time to elapse after the initial two coronary stenosis periods were completed. In animals in groups B, C, and D, we waited 5 minutes after the initial periods of coronary shunt stenosis and release and then performed a 5-minute complete coronary occlusion followed by reperfusion for 30 minutes. 4) Reperfusion control (C2), and 5) reperfusion graded coronary shunt stenosis (S2), again to produce moderate flow reductions, as nearly as possible equal in degree to the initial preocclusion flow reductions. As a guide to achieving equality of moderate flow reductions, we used the Doppler velocimeter signal and the distal coronary arterial pressure to match this postocclusion coronary shunt flow reduction to the earlier preocclusion coronary stenosis flow reduction. (Whether an actual match was achieved was determined by the microsphere perfusion data; see below.)

In each coronary stenosis experimental condition, after a 2-minute period of stabilization, we recorded the aortic and coronary arterial pressure, coronary arterial flow velocity, and M-mode echocardiographic signals or sonomicrometer signals followed by the injection of microspheres into the left atrium. The flow reduction in each individual coronary stenosis was limited to 4 minutes.

To briefly summarize the experimental protocols, control group (A) consists of control 1, shunt stenosis 1, 30 minutes’ elapsed time, no intervention, control 2, and shunt stenosis 2. Intervention groups

FIGURE 1. Illustrative recordings of the hemodynamic effects of repeated episodes of carotid-coronary shunt stenosis. Equivalent degrees of coronary arterial flow reductions were achieved during two episodes of coronary stenosis, as demonstrated by equal declines in Doppler-measured coronary flow and in coronary pressures.
(B, C, and D) consist of control 1, shunt stenosis 1, intervention (5 minutes of complete shunt occlusion and 30 minutes of reperfusion), superoxide dismutase and catalase infusion in group D, control 2, and shunt stenosis 2.

**Effect of Oxygen Free Radical Scavengers (Groups C and D)**

Our intention was to show that the sensitization phenomenon could be prevented by administration of oxygen free radical scavengers during the occlusion-reperfusion sequence. To do this, we divided the animals in this set of experiments into two groups. Group C (eight dogs) underwent a protocol identical to group B in the previous studies: a preocclusion control (C1), a preocclusion shunt stenosis of moderate degree (S1), a 5-minute complete coronary occlusion followed by 30 minutes of reperfusion, a reperfusion control (C2), and a reperfusion moderate shunt stenosis (S2) where the S2 perfusion reduction was intended to match the S1 reduction as closely as possible with the Doppler velocimeter and distal coronary arterial pressures as guides (but the definitive match depended on the microsphere-determined perfusion, as before). Hemodynamic recordings and microsphere injections were performed as in the earlier group B studies.

In 11 dogs (group D), we performed the same protocol except for the addition of oxygen free radical scavengers. Bovine superoxide dismutase (SOD; 5,000 units/min) and catalase (CAT; 20,000 units/min; Sigma Chemical, St. Louis, Missouri) was administered intravenously to each animal, beginning before the complete coronary occlusion and ending after the 30-minute reperfusion and control 2 periods.

**Measurements**

Hemodynamic parameters were calculated by averaging six consecutive beats: left ventricular systolic pressure, left ventricular end-diastolic pressure, peak positive and negative dP/dt, mean aortic pressure, and mean peripheral coronary pressure. Left ventricular relaxation was assessed by the time course of left ventricular pressure delay as proposed by Weiss et al.¹⁹ and Frederickson et al.²⁰ Left ventricular pressure was measured every 5 msec (t) for a period of 80 msec after left ventricular peak negative dP/dt. The diastolic left ventricular pressure values of natural log pressure (lnP) and t were fitted into a monoexponential relation by the least-squares method to the function lnP = At + B, where t = msec, A = slope of lnP versus t, and B = lnP of the highest pressure on the exponential relation. The time constant T = 1/−A. T1 represents the first 40 msec of isovolumic relaxation; T2 represents the last 40 msec. The ratio T2:T1 may be used as an index of left ventricular asynchrony during relaxation.

**TABLE 1. Global and Regional Left Ventricular Systolic Function Before and After 5-Minute Coronary Shunt Occlusion and 30-Minute Reperfusion**

<table>
<thead>
<tr>
<th>Group</th>
<th>Left ventricular end-diastolic thickness (mm)</th>
<th>Regional wall thickening (%)</th>
<th>Peak positive dP/dt (mm Hg/sec)</th>
<th>Left ventricular systolic pressure (mm Hg)</th>
<th>Mean aortic pressure (mm Hg)</th>
<th>Mean peripheral coronary artery pressure (mm Hg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>C1</td>
<td>C2</td>
<td>C1</td>
<td>C2</td>
<td>C1</td>
<td>C2</td>
</tr>
<tr>
<td>Group A</td>
<td>Mean</td>
<td>9.9</td>
<td>9.6</td>
<td>48.2</td>
<td>44.8</td>
<td>3,206</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>± SD</td>
<td>1.4</td>
<td>1.7</td>
<td>8.5</td>
<td>10.0</td>
<td>573</td>
</tr>
<tr>
<td>Group B</td>
<td>Mean</td>
<td>9.1</td>
<td>9.2</td>
<td>42.2</td>
<td>40.5</td>
<td>3,255</td>
</tr>
<tr>
<td>(n=9)</td>
<td>± SD</td>
<td>1.6</td>
<td>1.8</td>
<td>8.3</td>
<td>8.0</td>
<td>876</td>
</tr>
</tbody>
</table>

Group A, control and no coronary occlusion; Group B, 5-minute coronary occlusion and 30-minute reperfusion; C1, control 1, preocclusion; C2, control 2, after 5 minutes occlusion and 30 minutes reperfusion.

C1 vs. C2, p = NS (paired t test); group A vs. group B, p = NS (unpaired t test).
TABLE 2. Global and Regional Left Ventricular Diastolic Function Before and After 5-Minute Coronary Shunt Occlusion and 30-Minute Reperfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Peak negative dP/dt (mm Hg/sec)</th>
<th>T (msec)</th>
<th>Left ventricular end-diastolic pressure (mm Hg)</th>
<th>Wall thickness at 100 msec after end systole (% of end systole)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C1</td>
<td>C2</td>
<td>C1</td>
<td>C2</td>
</tr>
<tr>
<td>Group A</td>
<td>Mean</td>
<td>± SD</td>
<td>3,250</td>
<td>3,501</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td>50</td>
<td>926</td>
</tr>
<tr>
<td>Group B</td>
<td>Mean</td>
<td>± SD</td>
<td>3,752</td>
<td>3,378</td>
</tr>
<tr>
<td>(n = 9)</td>
<td></td>
<td></td>
<td>1,152</td>
<td>1,076</td>
</tr>
</tbody>
</table>

Group A, control; Group B, occlusion and reperfusion; C1, control 1, preocclusion; C2, control 2, after reperfusion. *p<0.01 (paired t test), C2 vs. C1; group A vs. group B, p = NS (unpaired t test).

Echocardiographic Measurements (Figure 2)

The epicardium was pulled adjacent to the ultrasonic transducer crystal by suction. We took as the epicardium the first visible ultrasonic reflector distal to the transducer artifact and arbitrarily measured with a caliper the wall thickness from the leading edge of the epicardium to the trailing edge of the endocardium-blood interface (Figure 2). Wall thickness measurements were obtained by averaging five successive cardiac beats. Wall thickness was measured by a separate observer who was not otherwise involved in the study. End diastole was arbitrarily defined as the electrical spike of the pacemaker signal; end systole was arbitrarily defined as the left ventricular peak negative dP/dt, corrected for the known display delay of 55 msec. Intraobserver variability for these M-mode echocardiographic wall thickness measurements in our laboratory is 2.6% (unpublished data).

Regional myocardial thickening was defined as ESTh—EDTh ÷ EDTh × 100. Regional diastolic myocardial relaxation was assessed by measuring wall thickness every 50 msec for the first 200 msec after end systole and normalized by considering end-systolic thickness as 100% and expressing diastolic thickness as percentage of the end-systolic value at each time point. Thus, the higher the percentage at any given moment in early diastole, the slower the relaxation. These early diastolic measurements were only made in the control (C1 and C2) recordings; during coronary shunt stenosis and (especially) occlusion, an early diastolic recoiling or apparent thickening occurs that makes instantaneous diastolic thickness measurements uninterpretable as a parameter of diastolic relaxation.

Sonomicrometer Measurements

The analog signals from the sonomicrometer tracings were used to measure end-diastolic and end-systolic wall thickness (endocardial-epicardial placement) or segmental length (side-by-side placement). End diastole was taken at the onset of the upstroke of the coronary arterial pressure signal; end systole was taken at the dicrotic notch of the coronary pressure signal.

Myocardial Perfusion

The risk area was identified by two different dyes (Van Giesen and Evans blue) injected at the end of the study simultaneously at identical pressures into

FIGURE 3. Plots of effects of 5 minutes of coronary occlusion and 30 minutes of reperfusion on regional early diastolic wall thinning (relaxation). In the control group, early diastolic wall thinning remained unchanged when assessed twice, 30 minutes apart. In the occlusion-reperfusion group, the reperfused myocardium thinned more slowly in early diastole, indicating impaired relaxation.
the left anterior descending coronary artery via the cannula and into the left main artery. The exact position of the M-mode echo probe had been previously marked with a fine suture when the probe was removed just before the end of the study so that the myocardial perfusion of that exact area could be determined. The echo probe was always shown by the dye injections to have been correctly placed in the coronary risk area. After 4 days of incubation in 3.7% formaldehyde, the hearts were cut into six slices perpendicular to the lower axis of the left ventricle. The six myocardial slices were cut according to the color code (left anterior descending coronary artery risk area, yellow; circumflex area, blue). The relative risk area was obtained by dividing the sum of the weights of the left anterior descending coronary artery pieces by the total weight of the left ventricle. The mean value of the risk area was 35.8±6.8% (±SD) and 38.5±5.6% (p = NS) for groups A and B, respectively.

Two 3-g samples were taken at the site marked by the suture where the suction M-mode probe had been placed (risk area), and two additional 3-g samples were taken from the nonischemic areas in the same slice. Regional transmural myocardial perfusion was calculated as previously described in detail21,22 and expressed as milliliters per minute per 100 grams. We then normalized flow; the perfusion of the to-be-ischemic area was divided by the flow to the nonischemic area. This ratio was then considered 100%, and subsequent flow ratios (ischemic:nonischemic) were divided by the initial ratio. We also determined endocardial:epicardial flow ratios whenever transmural perfusion was determined.21,22

Criteria for Data Inclusion

Our goal was to determine if an exaggerated deterioration of systolic function occurred when reperfused myocardium was subjected to subsequent moderate coronary flow reductions equal to initial preocclusion flow reductions. To accomplish this, we considered it essential to achieve matching of the moderate flow reductions (before and after complete coronary occlusion). Therefore, we arbitrarily insisted that the normalized perfusion of the ischemic area during the two graded coronary stenosis periods (S1 and S2) could not differ by more than 25% to fulfill the definition of an equivalent flow reduction. The flow reduction maneuver was, as noted, actually performed twice in the dogs in groups A and B: at the S1 point in the protocol and again at the S2 point. This was done in the hope of obtaining two sets of matching flow reductions from each animal. However, in only one animal in the intervention group and three in the control group did each of the two separate flow reductions at S1 actually match each of the two separate reductions at S2. In these four animals, the two S1 wall thickening and flow data points were averaged, as were the S2 points. In the majority of the group A and B animals, one S1 and one S2 point matched within the 25% criteria, and these data were included. In the group C and D dogs, we only performed one stenosis each at S1 and S2 stages. Data from four animals were excluded entirely because of unequal flow reductions (more than 25% variability) during the two graded coronary stenosis periods (S1 and S2). Two additional animals were excluded because of hemodynamic deterioration during the experiment. We report data from four animals in the control group A, nine in group B, eight in group C, and 11 in group D.

Statistical Analysis

Preocclusion versus reperfusion systolic data were compared within each group by two-tailed paired t tests during control and during coronary shunt stenosis (C1 vs. C2 and S1 vs. S2). Data from group A versus group B and group C versus group D were compared by unpaired t tests during control and during coronary shunt stenosis. Diastolic wall thinning in groups A and B was analyzed by analysis of variance with the Bonferroni modification to determine significance. All values are expressed in the tables as mean±SD.

Results

Hemodynamic, perfusion, echocardiographic, and sonomicrometer data are given in Tables 1–8 and Figures 1–5.

### Table 3. Regional and Global Left Ventricular Systolic Function During Equivalent Degrees of Coronary Shunt Stenosis Before and After 5-Minute Coronary Occlusion and 30-Minute Reperfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>End-diastolic wall thickness (mm)</th>
<th>Wall thickening (%)</th>
<th>Peak dp/dt (mm Hg/sec)</th>
<th>Left ventricular systolic pressure (mm Hg)</th>
<th>Mean aortic pressure (mm Hg)</th>
<th>Mean peripheral coronary artery pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td>Group A</td>
<td>Mean</td>
<td>8.9</td>
<td>8.6</td>
<td>17.2</td>
<td>14.4</td>
<td>3,002</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>± SD</td>
<td>1.3</td>
<td>1.6</td>
<td>15.9</td>
<td>13.1</td>
<td>473</td>
</tr>
<tr>
<td>Group B</td>
<td>Mean</td>
<td>8.9</td>
<td>8.4</td>
<td>12.1</td>
<td>−3.8†</td>
<td>2,752</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>± SD</td>
<td>1.5</td>
<td>1.8</td>
<td>17.1</td>
<td>14.3</td>
<td>548</td>
</tr>
</tbody>
</table>

Group A, control; Group B, occlusion and reperfusion; S1, initial moderate coronary shunt stenosis; S2, second moderate coronary shunt stenosis after an intervening period of occlusion-reperfusion.

* p<0.05, † p<0.01 (paired t tests), S2 vs. S1; Group A vs. Group B, p = NS (unpaired t test).
Groups A and B: Resting Data (Controls, C1 and C2) (Tables 1 and 2 and Figure 3)

All systolic and end-diastolic parameters assessed in this study were similar at the two control recordings, before occlusion (C1), and after occlusion and reperfusion (C2) in both the control (group A) and the occlusion-reperfusion (group B) groups of animals (Tables 1 and 2). Early diastolic regional wall thinning was unchanged when comparing C1 and C2 in the control animals (Figure 3), but diastolic wall thinning was slower (impaired) when C2 versus C1 were compared in the reperfused animals (group B animals) (Table 2 and Figure 3). Global left ventricular diastolic parameters (dP/dt, T, left ventricular end-diastolic pressure) were similar during control measurements (C1 and C2) in both groups of animals.

Groups A and B: Coronary Shunt Stenosis (S1 and S2)—Hemodynamic Effects in Systole (Table 3)

In both groups of animals, aortic and peripheral coronary pressures were similar during the moderate degrees of coronary arterial flow reduction (coronary shunt stenosis) (Table 3). In the control group (group A), similar flow reductions before (S1) and after (S2) 30 minutes of elapsed time (no occlusion) caused similar reductions of peak positive dP/dt, left ventricular systolic pressure, and left ventricular end-systolic pressure. In the occlusion-reperfusion group (B), there was a small fall of peak positive dP/dt when S2 is compared with S1; no other changes in systolic hemodynamic parameters occurred (Table 3).

Groups A and B: Coronary Shunt Stenosis—Hemodynamic Effects in Diastole (Table 4)

In the control group (group A), similar changes of global and regional indexes of left ventricular diastolic relaxation (peak negative dP/dt, time constants) were observed in response to the two moderate flow reductions (S1 and S2). In contrast, in the group B dogs, diastolic relaxation became significantly slower in response to a second moderate coronary arterial flow reduction after the occlusion-reperfusion sequence, as demonstrated by the decrease of peak negative dP/dt and the increase of T (Table 4). The change of the T2:T1 ratio suggests that left ventricle relaxation becomes asynchronous during the second "challenge" coronary occlusion after reperfusion in the group B (intervention) dogs.

Groups A and B: Coronary Shunt Stenosis (S1 and S2)—Myocardial Perfusion and Wall Thickening (Tables 3, 5, and 6 and Figure 4)

Equivalent coronary arterial flow reductions during S1 and S2 (coronary shunt stenosis) in the control dogs (group A) caused similar reductions of systolic wall thickening. In contrast, however, in the group B animals subjected to a sequence of complete coronary occlusion (5 minutes) and reperfusion (30 minutes), an exaggerated impairment of regional thickening occurred in response to the moderate coronary flow reduction after reperfusion (S2). In each animal in this group, regional thickening was lower during S2 than during S1; in three dogs, systolic wall thinning occurred during S2 when it had not occurred during S1. The individual data points of systolic wall thickening are given in Table 6.

Groups C and D: Effect of Oxygen Free Radical Scavengers

The hemodynamic and sonomicrometric data from groups C and D are presented in Table 7, and the perfusion data is given in Table 8. The endocardial-epicardial sonomicrometric data are illustrated in Figure 5. Although there were eight dogs in group C, technically adequate endocardial-epicardial somatic signals were obtained in only six dogs, as indicated in Figure 5, and side-by-side somatic signals were obtained in only seven dogs. Adequate signals were outlined in all 11 of the group D dogs (Figure 5).

In the group C animals, the C2 perfusion during the two levels of moderate coronary stenosis (S1 and S2) matched almost exactly (Table 8): S2, 36.1 ± 12.9 ml/min/100 g; S1, 36.6 ± 9.8 ml/min/100 g. The endocardial-epicardial sonomicrometric data showed that regional function did not entirely recover after the occlusion-reperfusion sequence (C1, 1.8 ± 0.8 mm; C2, 1.2 ± 0.5 mm, p<0.05) (Table 7). This differs from the echocardiographic results.

Table 4. Global Left Ventricular Diastolic Function During Equivalent Degrees of Coronary Shunt Stenosis Before and After 5-Minute Coronary Occlusion and 30-Minute Reperfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Peak negative dP/dt (mm Hg/sec)</th>
<th>T (msec)</th>
<th>T2:T1</th>
<th>Left ventricular end-diastolic pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 5)</td>
<td>Mean</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
</tr>
<tr>
<td></td>
<td>± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 9)</td>
<td>Mean</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
</tr>
<tr>
<td></td>
<td>± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Group A, control; Group B, occlusion and reperfusion; S1, initial moderate coronary shunt stenosis; S2, second moderate coronary shunt stenosis after an intervening period of occlusion-reperfusion.

*p<0.05, f*p<0.01 (paired t tests), S2 vs. S1; Group A vs. Group B, p = NS (unpaired t test).
Group A, control; Group B, occlusion and reperfusion; endo:epi, endocardial-epicardial ratio. All transmural perfusion expressed in milliliters per minute per 100 grams.

*p<0.05 (paired t test), S2 vs. S1.

The main finding of the present study is that brief ischemic and then reperfused myocardium responds to subsequent moderate coronary arterial flow reductions with an exaggerated impairment of regional systolic thickening.

Single episodes of 15 minutes of transmural ischemia are followed by prolonged depressions of regional systolic function; this phenomenon has been termed "stunning." The extent of recovery of regional myocardial function after a briefer 5-minute occlusion has been variably estimated by earlier studies. Using sonomicrometers, Heyndrickx et al. observed only partial recovery of segmental shortening within 30 minutes of reperfusion after a single period of 5 minutes of coronary occlusion. On the other hand, using echocardiography or other techniques to evaluate regional myocardial function, others have reported full recovery of briefly ischemic myocardium. In our study, after a 5-minute occlusion, regional systolic thickening at rest returned to the preocclusion levels by echocardiography but did show mild persisting reductions in contraction by sonomicrometry, and this is consistent with the previous reports of Heyndrickx et al. and Klener et al. Thus, sonomicrometry may be
somewhat more sensitive than echocardiography to subtle abnormalities of regional myocardial function at rest. Of importance is that by both echocardiography and sonomicrometry, after the occlusion-reperfusion sequence, a subsequent moderate reduction in coronary arterial flow produced an exaggerated impairment of regional systolic function compared with the response to similar moderate flow reductions before the occlusion-reperfusion sequence. We suggest that this exaggerated response of reperfused myocardium to subsequent coronary flow reductions can be considered a further manifestation of subtle stunning—posts ischemic myocardial dysfunction that becomes more evident when regional myocardial perfusion is reduced. Thus, the reperfused, stunned myocardium is sensitized to subsequent coronary arterial flow reductions.

There is other information available that suggests that myocardium subjected to brief coronary occlusion plus reperfusion responds abnormally to subsequent coronary flow reductions. In a related experiment, we found that the time course of the development of systolic thinning (dyskinesis) in response to a complete coronary occlusion was different in reperfused versus normal myocardium; canine myocardium previously subjected to an occlusion-reperfusion sequence developed overt dyskinesis within 10 seconds of a second coronary occlusion, whereas normal canine myocardium did not manifest systolic thinning until 20 seconds after coronary occlusion. These results are consonant with the present study.

Another indicator of myocardial dysfunction after an occlusion-reperfusion sequence was abnormal early diastolic regional relaxation. This was seen at rest (in the second control recording) as an abnormality of regional early diastolic thinning (Figure 3) and during the second coronary stenosis as slower, asynchronous global diastolic relaxation (Table 4). These data are in agreement with other studies reporting abnormal diastolic function after transient ischemia. Many clinical studies have shown that ventricular relaxation abnormalities are sensitive markers of coronary artery disease in the absence of systolic dysfunction.

What is the mechanism of the myocardial dysfunction we demonstrated? Because end-diastolic wall thickness did not increase, myocardial edema did not develop after the occlusion-reperfusion sequence. Heart rate was maintained constant by creating heart block and pacing, and blood pressure changed little. Thus, myocardial oxygen requirements were not substantially different before and after the occlusion-reperfusion sequences.

Coronary occlusion-reperfusion sequences generate oxygen free radicals. Recent studies have shown that administration of the oxygen free radical scavengers superoxide dismutase and catalase markedly enhanced the recovery of myocardial segment shortening in overtly dysfunctional (stunned) post-ischemic and reperfused canine myocardium.

Table 6. Regional Left Ventricular Systolic Function During Control and Coronary Shunt Stenosis—Individual Data Points

<table>
<thead>
<tr>
<th>Group</th>
<th>Dog</th>
<th>C1</th>
<th>S1</th>
<th>C2</th>
<th>S2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>(n=9)</td>
<td>1</td>
<td>40</td>
<td>-1</td>
<td>51</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
<td>27</td>
<td>34</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>2</td>
<td>40</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>23</td>
<td>40</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>35</td>
<td>59</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>48.2</td>
<td>17.2</td>
<td>44.8</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>± SD</td>
<td>8.5</td>
<td>15.9</td>
<td>10.0</td>
<td>13.1</td>
<td></td>
</tr>
</tbody>
</table>

Group A, control; Group B, occlusion and reperfusion; C1, control 1; S1, coronary stenosis 1; C2, control 2; S2, coronary stenosis 2.

*p<0.01, S2 vs. S1.

Table 7. Regional Systolic Function Before and After 5-Minute Coronary Shunt Occlusion and 30-Minute Reperfusion—Effect of Oxygen Free Radical Scavengers

<table>
<thead>
<tr>
<th>Group</th>
<th>Regional wall thickening [endo:epi sonomicrometers (mm)]</th>
<th>Segmental shortening [side-by-side sonomicrometers (mm)]</th>
<th>Peripheral coronary artery pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C1</td>
<td>S1</td>
<td>Ocl</td>
</tr>
<tr>
<td>Group C</td>
<td>Mean</td>
<td>1.8</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>± SD</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Group D</td>
<td>Mean</td>
<td>1.3</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>± SD</td>
<td>0.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

All transmural perfusion expressed in milliliters per minute per 100 grams.

Group C, 5-minute coronary occlusion and 30-minute reperfusion; Group D, coronary occlusion and 30-minute reperfusion and superoxide dismutase and catalase; C1, control 1; S1, coronary stenosis 1; Ocl, coronary occlusion; C2, control 2; S2, coronary stenosis 2.

*p<0.05 (paired t test), C2 vs. C1 or S2 vs. S1; †p<0.05, †p<0.01 (unpaired t tests), Group C vs. Group D.
This suggested that such free radicals might be responsible for the abnormal response to postreperfusion moderate flow reductions we have shown. To demonstrate this, we undertook experiments with superoxide dismutase and catalase. We found that these oxygen free radical scavengers prevent the exaggerated impairment of regional function in response to a postreperfusion flow reduction. This strongly suggests that oxygen free radical-mediated myocardial toxicity is the mechanism of the sensitization phenomenon we have described. This supports our suggestion that this sensitization phenomenon can be considered an extension of the concept of myocardial stunning because the latter may also be attributable to oxygen free radical toxicity.29-31

The relation between myocardial perfusion and function during progressive coronary stenosis has been studied by several investigators, who have reported the relation to be mildly or markedly curvilinear and to vary by layer (inner vs. outer myocardium).32-34 Our finding of an altered response to moderate coronary arterial flow reduction after a sequence of coronary artery occlusion-reperfusion suggests that the entire myocardial function-perfusion relation may be altered in reperfused myocardium. Defining the extent of such alterations and the shape of the new dyskinesis-perfusion relation would be of great interest but is beyond the scope of the present study.

The control myocardial perfusion levels in group A and B dogs in our study were low. There was no evidence of ischemia at this point in the study. The systolic wall thickening was normal; the endocardial:epicardial perfusion ratios exceeded 1.0; and further changes in hemodynamics, wall thickening, and perfusion occurred in response to the subsequent coronary stenosis. The paced heart rate of 90 beats/min is relatively low for an open-chest dog, and the systolic pressures were under 100 mm Hg. Relatively low heart rates and blood pressures would tend to reduce myocardial oxygen requirements, and myocardial perfusion would be correspondingly lower.

Although we made every effort to achieve equivalent moderate flow reductions when the coronary shunt was constricted, Table 5 shows that the second coronary shunt stenosis (S2) in the group B dogs yielded a transmural perfusion about 24% lower than the first stenosis (S1) in that group, and the S2 endocardial:epicardial flow ratio was significantly lower than that of S1. Thus, somewhat greater ischemia was induced by the second coronary stenosis in the group B dogs; we cannot entirely exclude this as a contributing cause of the exaggerated response to the second stenosis seen in that group of dogs. Note, however, that lower transmural perfusion and endocardial:epicardial ratios were seen during the second coronary stenosis of the control group A dogs, which did not produce the exaggerated impairment of wall thickening seen in the intervention group. Furthermore, in the group C dogs, we

### Table 8

<table>
<thead>
<tr>
<th>Group</th>
<th>Transmural perfusion</th>
<th>Endo:epi</th>
<th>Transmural perfusion</th>
<th>Endo:epi</th>
<th>Transmural perfusion</th>
<th>Endo:epi</th>
<th>Transmural perfusion</th>
<th>Endo:epi</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>Mean 73.7 0.94</td>
<td></td>
<td>Mean 36.6 0.80</td>
<td></td>
<td>Mean 95.9* 0.94</td>
<td></td>
<td>Mean 36.1 0.63</td>
<td></td>
</tr>
<tr>
<td>(n=8)</td>
<td>± SD 22.1 0.09</td>
<td></td>
<td>± SD 9.8 0.39</td>
<td></td>
<td>± SD 23.4 0.21</td>
<td></td>
<td>± SD 12.9 0.30</td>
<td></td>
</tr>
<tr>
<td><strong>Coronary stenosis 1</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>Mean 67.2 0.99</td>
<td></td>
<td>Mean 33.6 0.84</td>
<td></td>
<td>Mean 105.7 0.82</td>
<td></td>
<td>Mean 34.0 0.86</td>
<td></td>
</tr>
<tr>
<td>(n=11)</td>
<td>± SD 17.2 0.08</td>
<td></td>
<td>± SD 13.7 0.33</td>
<td></td>
<td>± SD 78.1 0.07</td>
<td></td>
<td>± SD 10.5 0.25</td>
<td></td>
</tr>
<tr>
<td><strong>Control 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>Mean 67.2 0.99</td>
<td></td>
<td>Mean 33.6 0.84</td>
<td></td>
<td>Mean 105.7 0.82</td>
<td></td>
<td>Mean 34.0 0.86</td>
<td></td>
</tr>
<tr>
<td>(n=11)</td>
<td>± SD 17.2 0.08</td>
<td></td>
<td>± SD 13.7 0.33</td>
<td></td>
<td>± SD 78.1 0.07</td>
<td></td>
<td>± SD 10.5 0.25</td>
<td></td>
</tr>
<tr>
<td><strong>Coronary stenosis 2</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*Group C, 5-minute coronary occlusion and 30-minute reperfusion; Group D, 5-minute coronary occlusion and 30-minute reperfusion and superoxide dismutase and catalase; endo:epi, endocardial:epicardial ratio.

*p<0.01 (paired t test), C2 vs. C1; Group C vs. Group D, p = NS (unpaired t test).

**FIGURE 5.** Bar charts of effects of oxygen free radical scavengers on the sensitization phenomenon, with sonomicrometers (endocardial-epicardial orientation). In the occlusion-reperfusion group (group C, left panel), an exaggerated decline in systolic thickening in response to a coronary shunt stenosis occurred after a sequence of coronary occlusion-reperfusion (sensitization). In the animals receiving oxygen free radical scavengers (group D, right panel), this exaggerated decline did not occur. Thus, oxygen free radical scavengers blocked the sensitization phenomenon.
achieved excellent matching of transmural perfusion during the two episodes of shunt stenosis, and regional contraction was again impaired during the second postreperfusion stenosis (Tables 7 and 8 and Figure 4). Thus, we believe that the intervention itself, the coronary occlusion-reperfusion sequence, is the primary cause of the exaggerated segmental contraction abnormality in response to subsequent moderate transmural perfusion reductions—sensitization.

The duration of the occlusion-reperfusion sequence may be important in inducing the sensitization phenomenon; some critical duration of flow interruption may be necessary. Because we studied only one arbitrary time sequence—5 minutes of coronary occlusion and 30 minutes of reperfusion—our study does not answer this question. Very brief coronary flow interruptions (up to 1 minute) occur during the clinical procedure of percutaneous transluminal coronary angioplasty; after the cessation of such brief flow interruptions, global and regional systolic function return rapidly to normal,35,36 but posts ischemic regional diastolic abnormalities (increased regional stiffness) may persist.35

Our experimental model is an artificial one and, of necessity, is rather unphysiological; the results cannot be directly transposed to the clinical situation. Nevertheless, it is known that patients with angina, especially vasospastic, may undergo repeated episodes of ischemia-reperfusion, and these episodes may occur in clusters.37–39 If our experiments have any clinical applicability, they suggest that such repeated ischemia-reperfusion episodes may have the potential to cause declines in regional function out of proportion to the perfusion reductions encountered. Clinical studies will be necessary to show that such a phenomenon actually occurs.

Acknowledgments

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