Protection by Superoxide Dismutase From Myocardial Dysfunction and Attenuation of Vasodilator Reserve After Coronary Occlusion and Reperfusion in Dog


Previous studies indicate impairment of coronary arterial ring relaxation and loss of coronary vasodilator reserve after coronary artery occlusion and reperfusion. These changes are mediated in part through loss of endothelium-derived relaxing factor (EDRF) and/or myocardial neutrophil accumulation. To examine if superoxide dismutase (SOD), a scavenger of superoxide radicals, would modify the diminished coronary vasodilator reserve after temporary coronary occlusion in the intact animal, open-chest mongrel dogs were subjected to 1 hour of circumflex (Cx) coronary artery occlusion followed by 1 hour of reperfusion and treated with saline or SOD. Before Cx occlusion, coronary blood flow increased, and vascular resistance decreased (both \( p < 0.01 \)) in response to EDRF-dependent vasodilator acetylcholine as well as EDRF-independent vasodilator nitroglycerin. After Cx reperfusion, resting Cx coronary blood flow and vascular resistance were similar to the preocclusion values. In the saline-treated animals, there was evidence of myocardial dysfunction, which was measured by segmental shortening (\(-6 \pm 2\% \) vs. \(10 \pm 2\% \)). Furthermore, increase in Cx coronary blood flow and reduction in vascular resistance in response to both vasodilators were significantly \( (p < 0.01) \) impaired; these occurrences suggested loss of coronary vasodilator reserve. Myocardial histology showed extensive capillary plugging by neutrophils in the Cx-supplied myocardium. Myocardial myeloperoxidase activity, an index of neutrophil infiltration, was also increased in the Cx compared with the left anterior descending coronary artery region \((p < 0.02)\). Treatment of dogs with SOD, started at the end of Cx occlusion and continued during reperfusion, exerted significant \( (p < 0.01) \) protective effect against reperfusion-induced attenuation of coronary vasodilator reserve in response to both acetylcholine and nitroglycerin. Loss of myocardial function (segmental shortening \(5 \pm 1\% \) vs. \(10 \pm 1\%) \) was less than in the saline-treated animals \((p < 0.01)\). Cx region–myocardial neutrophil accumulation and myeloperoxidase activity were also less \((p < 0.02)\) in the SOD-treated than in the saline-treated dogs. These observations suggest that coronary reperfusion impairs coronary vasodilator reserve in intact dogs. This impairment can be modified by treatment of animals with SOD before reperfusion. Capillary plugging by neutrophils may contribute to the altered coronary vasodilator reserve observed in the immediate postreperfusion period, and SOD modifies this reperfusion-induced impairment.

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**Endothelium participates in the maintenance of coronary arterial tone by releasing vasodilator and vasoconstrictor substances. One of the most potent vasodilator substances, endothelium-derived relaxing factor (EDRF), is released from the endothelial cells. Gryglewski and coworkers have shown that superoxide anion is involved in the breakdown of EDRF. Furchgott and**


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Zawadzki first described the obligatory role of endothelial cells in vascular relaxation evoked by acetylcholine (ACh) mediated by release of EDRF. Subsequent studies showed that the coronary artery ring relaxation by ACh or thrombin is impaired when dog coronary arteries are subjected to total occlusion followed by reperfusion. The role of reperfusion in extension of myocardial injury has recently been reviewed. It is apparent that reperfusion, although somewhat beneficial in the salvage of ischemic myocardium, may also be detrimental by reducing coronary relaxation. Entry of neutrophils into the myocardium during reperfusion and occlusion of capillaries may reduce microvascular cross-sectional area. Release of superoxide anions and other oxygen free radicals by neutrophils and endothelial cells in the early stages of reperfusion may also relate to the detrimental effects of reperfusion. Some investigators have indeed shown beneficial effect of scavengers of oxygen free radicals on arrhythmias and infarct size in animals subjected to coronary occlusion followed by reperfusion. However, such benefit has not been observed by others. These variable results may relate, in part, to the vagaries of canine coronary circulation and also to different protocols used in these studies.

Diminished coronary vasodilator reserve after temporary coronary occlusion in the dog has been well documented. This reduction in vasodilator reserve may be due to the loss of EDRF, reduction in microvascular cross-sectional area by accumulation of neutrophils, loss of myocardial function, and to a variety of other factors. The present study was designed to examine if use of a scavenger of superoxide anion, superoxide dismutase (SOD), would modify reperfusion-induced myocardial dysfunction and attenuation of coronary vasodilator reserve in the anesthetized dogs.

Materials and Methods

Experimental Preparation

Twelve healthy adult mongrel dogs of either sex weighing 20–32 kg (average, 25 kg) were used for this study. The animals were anesthetized with intravenous sodium pentobarbital at a dose of 30 mg/kg body wt. Small maintenance doses of the anesthetic agent were administered periodically when needed during the experiment. Once the animal was anesthetized, auffed endotracheal tube was inserted, and respiration was maintained with an intermittent positive-pressure respirator (Harvard Apparatus, South Natick, Massachusetts). The rate and volume per stroke of the respirator were adjusted to maintain arterial blood gases within the physiological range (pH, 7.35–7.45; Pco2, 30–40 mm Hg; and Pao2, 85–100 mm Hg). The heart and great vessels were exposed through the fifth intercostal space. The pericardium was opened and reflected to form a cradle for suspending the heart. Approximately 2 cm of the left anterior descending (LAD) and circumflex (Cx) coronary arteries were dissected free from the adjacent tissues. Flows in the two arteries were measured with a dual-channel 20 MHz ultrasonic Doppler flowmeter (Crystal Biotech, Holliston, Massachusetts) and with rigid flow probes with an inside diameter ranging from 2.0 to 3.5 mm. Probes were carefully matched to the diameter of the artery in each case and were chosen so that the probe slightly constricted the vessel. The Doppler flowmeter and probes were calibrated by matching the output signal to a calibrated electromagnetic flowmeter (model BL 613, Biotronex, Kensington, Maryland) signal. A pressure catheter (Millar, Houston, Texas) was passed through a carotid artery and positioned so that the tip was in the ascending aorta. A small Teflon 3F catheter was inserted into the first diagonal branch of the LAD and advanced to the left main coronary artery. Blood flow in LAD and Cx coronary arteries increased equally after a 50 μg bolus injection of nitroglycerin (NTG); this increase indicated catheter position in the left main coronary artery. The catheter’s position was also confirmed at the termination of the experiment by opening the vessel. A small plastic occluder was positioned around the Cx artery and used for total occlusion of the vessel.

A pair of miniature ultrasonic crystals (2 mm diameter) were positioned in the midmyocardium in the region supplied by the Cx artery. In implanting the crystals, a small tract was created with an 18-gauge needle, a transparent Teflon tube was placed around the wire leading from the crystals to provide support, and the crystals were then pushed to the desired depth (about 8 mm from the epicardium) as marked by a ligature on the wire. The orientation of the crystals, which were separated by 1.0–1.5 cm, was aligned so that the optimal acoustic signal was obtained from the receiver crystal. After positioning, the Teflon tube was removed. Although precise myocardial fiber angles at the sites of crystal implantation could not be obtained, the depth of the crystals, their orientation transverse to the long ventricular axis, and their proximity to the minor equator made it likely that they lay generally parallel to the muscle fiber. End-diastolic segment length (EDL) and end-systolic segment length (ESL) were measured at aortic valve opening and closure, respectively, which was determined from the ascending aortic pressure waveform. Segment length change or shortening was obtained as follows: EDL – ESL / EDL * 100. Lead II of the electrocardiogram was monitored to assess heart rate, arrhythmias, and S-T segment alterations.

Two of 10 dogs were anesthetized and instrumented in the same manner as the other 10 dogs and served as sham controls.

Experimental Protocol

After a stabilization period of at least 15 minutes, heart rate, ascending aortic pressure, coronary blood
flows (LAD and Cx), and segment length change were measured.

ACh or NTG was then infused into the left main coronary artery in a random fashion over 10 seconds. The amount of ACh, dissolved in saline, varied from 0.125 to 1 μg, always in a volume of 0.2 ml. NTG (6.25–50 μg), dissolved in 0.2 ml saline, was administered in a manner similar to ACh. In all experiments, coronary blood flows and arterial pressure were allowed to return to baseline before administration of ACh or NTG. The vehicle (normal saline) was frequently infused (0.2 ml) over 10 seconds to confirm only minimal (<10%) change in coronary blood flow.

The animals were then randomly divided into two groups. Five dogs were subjected to total Cx coronary occlusion for 1 hour followed by reperfusion for 1 hour (Figure 1). These animals received saline throughout the period of coronary occlusion and reperfusion. Five other dogs underwent a similar procedure except that at 50 minutes of total Cx occlusion, 2 mg/kg recombinant-human SOD (Pharmacia, Uppsala, Sweden) was administered intravenously over 5 minutes. Another 4 mg/kg SOD was gradually infused over 30 minutes. Cx coronary artery reperfusion was initiated after 1 hour of occlusion. Thus, SOD infusion was continued for 20–25 minutes into reperfusion (Figure 2).

To prevent serious arrhythmias and ventricular fibrillation, reperfusion was accomplished in all animals by gradually releasing the occluder over approximately 15 minutes (Figures 1 and 2). After 1 hour of reperfusion, ACh and NTG administration was repeated in both groups of animals. If the mean arterial pressure after reperfusion was lower than in the preocclusion state, phenylephrine was administered intravenously to increase the mean arterial pressure to the pre-Cx occlusion value.

The two control dogs were subjected to sham coronary occlusion and reperfusion and were given ACh and NTG in the same fashion as the other dogs.

**Histology**

After the completion of the experiments, dogs were killed by rapid injection of excess of potassium chloride, and the hearts were quickly removed. Hearts were placed in 10% formalin. Five equal-thickness transverse sections were cut. Histological processing was done by conventional methods, and sections were stained with hematoxylin and eosin. The histological sections were examined for intensity and extent of neutrophil deposition in the LAD- and Cx-supplied regions. The intensity and severity were arbitrarily graded from grade 0 to grade 4, such that grade 0 intensity would imply absence of neutrophils in the myocardium and grade 4 would imply dense neutrophil infiltration. Grade 1 extent would suggest local area/s of neutrophil infiltration, whereas grade 4 extent would suggest very extensive neutrophil infiltration between myocytes as
Myocardial Myeloperoxidase Assay

As a specific enzymatic marker of neutrophil infiltration into the reperfused myocardium, approximately 500 mg left ventricular tissue (including endocardium) was removed from the center of the regions supplied by the Cx and LAD coronary arteries and thoroughly washed to remove any red blood cells. A sample of peripheral blood was collected in heparin (10 units/ml). The blood was diluted with normal saline (1:1, vol/vol) and layered over a ficoll-hypaque preparation (Histopaque 1.077, Sigma Chemical, St. Louis, Missouri). This preparation was centrifuged at 800g for 30 minutes at 25°C. The supernate containing the neutrophils was then centrifuged at 2,000g to obtain a pellet. The cells were then washed three times with Hank's Balanced Salt Solution and assayed for purity and viability, which were both greater than 90%. A modification of the method of Bradley et al.28 was used to measure myeloperoxidase activity in the myocardial sections and in neutrophils.21,22,29 The person performing the myeloperoxidase assay (D.L.L.) was also kept blinded as to the treatment of the animals and the histological assessment.

Plasma SOD Concentrations

Peripheral venous blood was collected in heparinized tubes before and for up to 120 minutes after SOD administration. Blood was centrifuged, and plasma was stored at −70°C for subsequent measurement of SOD concentrations, which were determined by Pharmacia Labs, Uppsala, Sweden.

Data Recording and Calculations

The hemodynamic variables, electrocardiograms, and segment length change were recorded continuously on a strip chart recorder (Gould, Cleveland, Ohio). Coronary blood flow just before NTG administration was used as an index of flow. Increase in blood flow was determined as follows: peak flow−rest flow/rest flow×100. Coronary vascular resistance was calculated as mean aortic pressure/mean coronary blood flow.

Statistical Analysis

All data presented are peak changes and are expressed as mean±SEM. Student's t test (for data) and repeated-measures ANOVA using absolute values for coronary flow and vascular resistance were used for statistical analyses. Post-Cx reperfusion values were compared with pre-Cx occlusion values.
values in each group, and post-Cx reperfusion values were compared in the two groups of animals. A value of \( p < 0.05 \) was considered significant.

All data analyses were done by the Division of Biostatistics of the University of Florida, Gainesville with the help of Statistical Analysis System.

**Results**

Before Cx occlusion, coronary blood flow (Cx and LAD) increased in response to ACh and NTG in a dose-dependent fashion in all animals \( (p < 0.01) \) (Figures 3 and 4). There was no significant difference in response to ACh and NTG in dogs that subsequently received SOD or saline. Accordingly, data on coronary blood flow in all dogs were pooled (Figure 5). Coronary vascular resistance decreased \( (p < 0.01) \) in LAD and Cx, and the reductions were dependent on the amount of ACh or NTG infused (Tables 1 and 2). Heart rate, arterial pressure, and myocardial segment length were not significantly affected by either of the two agents at the peak coronary blood flow effect.

**Saline-Treated Animals**

After 1 hour of Cx occlusion and 1 hour of reperfusion, mean arterial pressure was lower than in the pre-Cx occlusion state in two of five animals. As per protocol, mean arterial pressure was increased in these animals by infusion of phenylephrine to pre-Cx occlusion values. Resting hemodynamic variables, including coronary blood flows, coronary vascular resistance, heart rate, and mean arterial pressure were similar to pre-Cx occlusion values in all animals before administration of ACh or NTG (Table 3). However, EDL and ESL in Cx-supplied myocardium were increased, and EDL was always smaller than ESL; this difference indicated "paradoxical" motion of the left ventricular wall (Table 3, Figures 1 and 3). This resulted in a significant change in myocardial segmental shortening \(-6 \pm 2\) vs. \(10 \pm 2\%), \( p < 0.01\).

Increase in Cx coronary blood flow in response to both ACh and NTG was significantly \( (p < 0.01) \) attenuated (Figures 3 and 5). Reduction in Cx coronary vascular resistance was also significantly \( (p < 0.01) \) blunted compared with that before Cx occlusion (Table 1). In contrast, increase in LAD coronary blood flow in response to both ACh and NTG was preserved (Figure 3). LAD coronary vascular resistance also decreased and was not affected by Cx occlusion-reperfusion (Table 1).
**SOD-Treated Animals**

Administration of SOD had no effect on systemic or coronary hemodynamics. The plasma SOD concentration peaked at 10 minutes after administration and gradually declined to baseline levels at approximately 60 minutes (Figure 6).

After Cx reperfusion, mean arterial pressure was lower than in the preocclusion state in two of five animals in this group as well, and it was increased to pre-Cx occlusion values by administration of phenylephrine. Overall, before administration of ACh or NTG, heart rate, mean arterial pressure, and coronary blood flows were comparable to pre-Cx occlusion values. Myocardial EDL and ESL in the Cx-supplied region were increased, and the segmental shortening was significantly less (5±1% vs. 10±1%, p<0.01) than before Cx occlusion (Table 4). However, in contrast to the saline-treated animals (Table 3), paradoxical motion of Cx-region myocardium was not observed (Figures 2 and 4).

Administration of ACh and NTG caused increase in coronary blood flow and decrease in coronary vascular resistance in both LAD and Cx regions (Figure 4 and Table 2). Increase in coronary blood flow...
Effect of ACh and NTG in Sham Control Animals

were readministered 2-3 hours later (time of ACh fused Cx region were similar to that before Cx occlusion (Figures 4 and 5, Table 2). This is in marked contrast to saline-treated animals, in whom increase in coronary blood flow was significantly reduced in the reperfused Cx region (Figure 5 and Table 1).

Effect of ACh and NTG in Sham Control Animals

In two control dogs with sham coronary occlusion, ACh and NTG caused a dose-dependent increase in coronary blood flow and decrease in coronary vascular resistance that were similar to those in the other 10 dogs. When ACh and NTG were readministered 2-3 hours later (time of ACh and NTG administration in the first 10 animals), there were no major differences in coronary blood flow or vascular resistance responses; thus, the attenuated Cx blood flow response to ACh and NTG in saline-treated dogs was not related to deterioration of the preparation.

Histopathology

In the saline-treated animals, myocardial tissues in the Cx-supplied myocardial region showed evidence of early myocardial damage, that is, wavy fibers and cell separation, cell contracture, cytoplasmic eosinophilia, and intense (grade 3-4) neutrophil infiltration of the intercellular spaces. Intercellular edema was frequently observed. Capillary plugging by neutrophils with interspersed erythrocytes, mimicking rouleaux formation, was the most characteristic feature of the reperfused myocardium particularly in the subendocardial region (Figure 7). Neutrophils adhered to the endothelial lining of the

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### Table 1. Effects of Acetylcholine and Nitroglycerin on Left Anterior Descending and Circumflex Coronary Vascular Resistances in Saline-Treated Animals

<table>
<thead>
<tr>
<th></th>
<th>Cx coronary vascular resistance (mm Hg/ml/min)</th>
<th>LAD coronary vascular resistance (mm Hg/ml/min)</th>
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<tbody>
<tr>
<td></td>
<td>Preocclusion</td>
<td>Change (%)</td>
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<tr>
<td>Acetylcholine (μg)</td>
<td>0</td>
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<td></td>
<td>1.0</td>
<td>1.8±0.3</td>
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<tr>
<td>Nitroglycerin (μg)</td>
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<td>4.3±0.5</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
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<tr>
<td></td>
<td>12.5</td>
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<td>25.0</td>
<td>1.8±0.4</td>
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<td></td>
<td>50.0</td>
<td>1.7±0.2</td>
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Values are mean±SEM and are the summary of five experiments. Cx, circumflex; LAD, left anterior descending. *p < 0.01 compared with preocclusion value.

### Table 2. Effects of Acetylcholine and Nitroglycerin on Left Anterior Descending and Circumflex Vascular Resistances in Superoxide Dismutase-Treated Animals

<table>
<thead>
<tr>
<th></th>
<th>Cx coronary vascular resistance (mm Hg/ml/min)</th>
<th>LAD coronary vascular resistance (mm Hg/ml/min)</th>
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<tr>
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<td>0.5</td>
<td>1.8±0.3</td>
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<td></td>
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<td>1.5±0.2</td>
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<tr>
<td>Nitroglycerin (μg)</td>
<td>0</td>
<td>4.5±0.3</td>
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<tr>
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<td>12.5</td>
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<td></td>
<td>25.0</td>
<td>2.4±0.3</td>
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<td></td>
<td>50.0</td>
<td>2.1±0.3</td>
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Values are mean±SEM. Cx, circumflex; LAD, left anterior descending.
TABLE 3. Effects of Circumflex Occlusion and Reperfusion on Systemic and Coronary Hemodynamics in Saline-Treated Animals

<table>
<thead>
<tr>
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<th>Before Cx occlusion</th>
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</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>140±5</td>
<td>143±5</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>128±10</td>
<td>123±3</td>
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<tr>
<td>LAD coronary blood flow (ml/mm)</td>
<td>27±3</td>
<td>25±3</td>
</tr>
<tr>
<td>Cx coronary blood flow (ml/min)</td>
<td>31±2</td>
<td>34±3</td>
</tr>
<tr>
<td>End-systolic length (mm)</td>
<td>13.8±1.5</td>
<td>18.4±1.6*</td>
</tr>
<tr>
<td>End-diastolic length (mm)</td>
<td>15.3±1.5</td>
<td>17.3±1.6*</td>
</tr>
<tr>
<td>Change in myocardial segment length (%)</td>
<td>10±2</td>
<td>-6±2*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Cx, circumflex; LAD, left anterior descending. *p<0.01 compared with pre-Cx occlusion.

arterioles and completely occluded several vascular lumina; they were also present between myocytes throughout the reperfused area (extent, grade 3–4). Similar changes were not observed in the LAD-supplied myocardium or the myocardium of sham control animals (intensity and extent of neutrophil infiltration, grade 0–1).

In comparison with the saline-treated animals, histology showed markedly less neutrophil accumulation in the myocardium, and fewer capillaries were plugged with neutrophils in the SOD-treated animals (intensity of neutrophil infiltration, grade 0–1; extent, grade 1–2). However, myocardial injury with cell separation and contraction bands was still evident (Figure 8).

Myeloperoxidase Assay

Neutrophil-specific myeloperoxidase activity in the Cx reperfused region (range, 0.14–0.25 units/100 mg tissue; mean, 0.21±0.03 units/100 mg tissue; equivalent to 3.97±10^5 neutrophils) was three to five times greater (p<0.02) than in the LAD region (range, 0.02–0.07; mean, 0.05±0.01 units/100 mg tissue; equivalent to 0.90×10^5 neutrophils) in the saline-treated animals. In the SOD-treated animals, myeloperoxidase activity was significantly (p<0.005) decreased in the reperfused Cx region (range, 0.03–0.10 units/100 mg tissue; mean, 0.08±0.02 units/100 mg tissue; equivalent to 1.52×10^5 neutrophils) compared with that in the saline-treated animals. In the patent LAD region, myeloperoxidase activity (range, 0.01–0.04 units/100 mg tissue; mean, 0.03±0.01 units/100 mg tissue; equivalent to 0.57×10^5 neutrophils) was similar to that in the saline-treated animals.

Discussion

Reperfusion of the myocardium after coronary occlusion may reduce infarct size and improve myocardial function.30,31 But many studies also point to a potentially detrimental effect of reperfusion on endothelial function, myocardial structure, and coronary vascular reactivity.4–7,21–23 Speciﬁcally, EDRF-dependent coronary arterial ring relaxation after reperfusion is impaired.4,5 Our studies show that EDRF-dependent ACh as well as EDRF-independent NTG-induced increase in coronary blood flow is significantly attenuated in the anesthetized animals after temporary coronary artery occlusion. The reduction in coronary vascular resistance in response to both vasodilators is similarly impaired. These observations indicate that the impairment in coronary vasodilation is not limited to the EDRF-dependent stimuli. Reduction in coronary vasodilator reserve that is measured by myocardial reactive hyperemic response is also evident in this setting.21,23

It is attractive to postulate that generation of superoxide radicals during reperfusion11–14 causes degradation of EDRF2 and accounts for the altered coronary arterial relaxation.4,5 Although loss of EDRF during reperfusion may not directly explain attenuated flow response to NTG, it is likely that endothelial injury permits adhesion of leukocytes during reperfusion and results in mechanical plugging of microvasculature and that flow response to NTG is secondarily diminished. Thus, loss of EDRF may indirectly diminish flow response to EDRF-independent vasodilators. Release of vasoconstrictor substance/s from the endothelium33 has also been described in this setting. Klein et al23 have suggested that myocardial dysfunction after coronary occlusion and reperfusion, and its extent, may relate to the loss of coronary vasodilator reserve. Any of these factors or their combination probably account for the decrease in coronary vasodilator reserve.34 It is noteworthy that changes in prostaglandin release do not play a role in reduction in coronary vasodilator reserve since inhibition of

![Figure 6](http://circres.ahajournals.org/)

**Figure 6.** Graph showing plasma superoxide dismutase (SOD) concentrations in SOD-treated animals.
prostaglandin synthesis does not modify this phenomenon.21

Our present study confirms previous observations that neutrophils appear in the reperfused canine myocardium in large numbers.8,9 Myocardial histology showed extensive neutrophil infiltration and plugging of large numbers of capillaries as well as tissue edema in the reperfused Cx region in contrast to the myocardium supplied by the patent LAD, which showed very little neutrophil infiltration and tissue edema. Threefold to fivefold increases in Cx myocardial myeloperoxidase activity also suggest presence of large numbers of neutrophils in the reperfused myocardium. Capillary plugging by neutrophils can mechanically reduce the microvascular cross-sectional area,8,9 and tissue edema can "fix" the arterioles. These factors may result in attenuated coronary vasodilator response. In addition, similar reduction in coronary blood flow response to EDRF-dependent vasodilator ACh as well as EDRF-independent vasodilator NTG, which may be similar to endogenous EDRF,25 suggests that mechanical obstruction in and around the coronary microvasculature plays an important role in the reduction in vasodilator reserve after coronary artery occlusion and reperfusion. Engler and colleagues8,9 indeed showed that reduction in neutrophil number in the dog before reperfusion significantly improves the "no-reflow" phenomenon and implied an important role of neutrophils in reperfusion-induced injury.

To examine the hypothesis that the release of superoxide anions by neutrophils and other sources during reperfusion is a factor in vascular injury,10 as well as in the breakdown of EDRF,2 we randomly treated a group of dogs with SOD or saline before as well as during the early phase of coronary reperfusion. With SOD administration, peak plasma concentrations were approximately 70 μg/ml at the time of reperfusion with gradual decline over 60 minutes. We observed a consistent and significant modification of reperfusion-induced reduction in coronary vasodilator reserve in these SOD-treated animals. This preservation of coronary vasodilator reserve was observed when either ACh or NTG was used as the vasodilator stimulus. Importantly, deterioration in myocardial function, as indicated by reduction in myocardial segmental shortening, in the reperfused Cx region was also significantly modified in the SOD-treated animals. It is quite likely that the improvement in coronary vasodilator reserve occurred as a result of dismutation of the superoxide anions and preservation of myocardial function with administration of SOD. It should however, be pointed out that the extent of myocardial ischemia and collateral blood flow, which determine coronary vasodilator reserve and systolic function,23,36,37 were not measured in these studies. Nonetheless, SOD-induced reduction in the extent of myocardial injury has previously been documented.15-18 Furthermore, preservation of coronary vasodilator reserve and segmental function identified in this study were much greater in SOD-treated animals than could be expected from a chance occurrence.

We were impressed by the presence of fewer neutrophils and correspondingly lower levels of myeloperoxidase in the reperfused myocardium in the SOD-treated compared with the saline-treated animals. The inhibitory effect of SOD on neutrophil chemotaxis (personal communication, R. Duque, MD, Department of Pathology, University of Florida) may have played an important role in these observations. Lack of capillary plugging by neutrophils probably contributed to the preservation of vasodilator reserve in response to EDRF-independent vasodilator NTG. This suggests that loss of EDRF during reperfusion is indirectly responsible for loss of NTG-induced vasodilation. Release of leukotriene B4, proteolytic enzymes, and superoxide anions from activated neutrophils are also important factors in the extension of myocardial injury after reperfusion.10,38 Decrease in neutrophil accumulation and superoxide anion generation in the reperfused region may explain the preservation of myocardial function in the SOD-treated animals.

There is ample evidence for the release of superoxide anions in the early phase of coronary reperfusion11-14 and its inhibition by SOD.39 Many investigators have demonstrated functional improvement of the "stunned" myocardium with SOD,40-44 which is observed after coronary reperfusion and thought to be related in part to the release of

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<tr>
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<td>End-diastolic length (mm)</td>
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<td>Change in myocardial segment length (%)</td>
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Values are mean±SEM. Cx, circumflex; LAD, left anterior descending.
*p<0.01 compared with pre-Cx occlusion.
Figure 7. Photomicrographs showing myocardial histology in patent left anterior descending (upper panel) and reperfused circumflex region (lower panel) from a representative saline-treated animal. Note myocardial cell swelling, cell separation and contraction bands, neutrophil infiltration between cells, and neutrophil plugging of capillaries after reperfusion in the circumflex region (hematoxylin and eosin stain). Original magnification, ×200.
FIGURE 8. Photomicrographs showing myocardial histology in patent left anterior descending (upper panel) and reperfused circumflex region (lower panel) in a representative superoxide dismutase-treated animal. Although the reperfused region shows myocardial injury, the neutrophil infiltration is markedly less than in the reperfused region of saline-treated animal (Figure 7, lower panel) (hematoxylin and eosin stain). Original magnification, ×200.
oxygen-derived free radicals. Our data on improved function of the reperfused Cx myocardium are in accordance with these observations. In previous studies from several laboratories, free radical scavengers were shown to limit myocardial injury in dogs subjected to coronary occlusion and reperfusion lasting up to 24 hours. However, results from other laboratories failed to demonstrate similar benefit of SOD on the size of myocardial infarct after coronary occlusion and reperfusion. The differences in the study protocols and the variations in coronary circulation of dog may account for the different results obtained in these studies. Most importantly, a 40-minute period of coronary occlusion used by Uraizee et al. may not result in injury to the myocardium caused by neutrophils; this injury is caused mainly by the "no-reflow" phenomenon. Similarly, myocardial injury after 3 hours of coronary occlusion in the dog, used by Gallagher et al., may not be amenable to recovery by SOD or other neutrophil inhibitory agents. Since the extent of myocardial injury in the dogs after coronary occlusion and reperfusion is dependent on vagaries of coronary circulation and other factors, we focused our studies on coronary vasodilator reserve and myocardial segmental function and consistently observed their impairment in the reperfused region in the saline-treated dogs subjected to 1 hour of Cx occlusion and 1 hour of reperfusion. Our present study demonstrates that SOD given before and during reperfusion prevents the detrimental effect of reperfusion on coronary vasodilator reserve as well as myocardial segmental function. Whether these beneficial effects of SOD, shown in the immediate postreperfusion period, are sustained during the subsequent period and relate to the cardioprotection by SOD as observed by Werns et al. and Ambrosio et al. is not known. However, the reduction in neutrophil accumulation and preservation of vasodilator reserve in the reperfused region with SOD treatment may have contributed to these observations.

In summary, we have documented attenuation of coronary vasodilator reserve and deterioration of myocardial function after reperfusion in the anesthetized dogs. The alteration in coronary vasodilator reserve is observed in response to both EDRF-dependent as well as independent vasodilators and is associated with plugging of capillaries by neutrophils in the reperfused myocardium. In this setting, administration of SOD appears to have beneficial effects on coronary vasodilator reserve and myocardial function, probably via inhibitory effect on myocardial neutrophil accumulation and/or preservation of EDRF.

References


KEY WORDS: acetylcholine • coronary vascular resistance • coronary vasodilator reserve • superoxide dismutase • coronary blood flow • endothelium-derived relaxing factor • nitroglycerin
Protection by superoxide dismutase from myocardial dysfunction and attenuation of vasodilator reserve after coronary occlusion and reperfusion in dog.

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