Comparative effects of nitroprusside and pinacidil on myocardial blood flow and infarct size in awake dogs with acute myocardial infarction

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ABSTRACT The effect of nitroprusside in limiting myocardial infarct was compared with that of pinacidil, a new antihypertensive agent with potent coronary vasodilator properties, in instrumented awake dogs subjected to 4 hr of left anterior descending coronary artery occlusion and 20 hr of reperfusion. Dogs were randomly assigned to receive intravenous normal saline, nitroprusside, or pinacidil beginning 40 min after the onset of coronary artery occlusion and continuing throughout the occlusion and the first hour of reperfusion. Nitroprusside and pinacidil were titrated to decrease mean aortic pressure by 25 mm Hg; normal saline had no effect on mean aortic pressure. Other systemic hemodynamic variables were not significantly altered by normal saline or nitroprusside, and myocardial blood flow did not change during normal saline infusion in normal and ischemic myocardium. In contrast, nitroprusside increased the blood flow and the endocardial/epicardial flow ratio in ischemic myocardium. This increase in ischemic myocardial blood flow was accompanied by a significant reduction in infarct size (40 ± 3% of region at risk vs 58 ± 4% in the normal saline group; p < .05). Pinacidil increased heart rate, cardiac output, and the peak rate of rise of left ventricular pressure. Furthermore, despite causing a threefold to fourfold increase in normal myocardial blood flow, pinacidil had no effect on either blood flow to ischemic myocardium or infarct size (57 ± 5%). The data indicate that the marked coronary vasodilator effect of pinacidil does not cause an increase in ischemic blood flow or a reduction in infarct size. Instead, infarct size is reduced by nitroprusside, suggesting that the latter agent is a preferred vasodilator in acute myocardial infarction.


THE USE OF vasodilators in the treatment of patients with myocardial infarction has received considerable interest. Although the reduction in preload and afterload with the resulting decrease in myocardial oxygen consumption is a clear benefit of such agents, the decrease in coronary perfusion pressure may potentially decrease blood flow to ischemic myocardium while the reflex increase in heart rate may further exaggerate ischemic injury. Nitroprusside is a prototype vasodilator that has been shown to improve left ventricular function in acute myocardial infarction. However, little is known regarding the effect of nitroprusside in limiting infarct size.

Nitroprusside dilates coronary arteries and has been shown to increase blood flow to both the normal and ischemic myocardium when it is infused to decrease arterial pressure 25 mm Hg from a hypertensive level to normal values. However, when arterial pressure is decreased further to 60 mm Hg below baseline with larger doses of nitroprusside in the hypertensive dogs, blood flow increases to the nonischemic myocardium and ischemic myocardial blood flow decreases. The latter phenomenon suggests a redistribution of coronary blood flow away from ischemic region to normal myocardium and is termed "coronary steal." A decrease in ischemic myocardial blood flow also has been shown during infusion of nitroprusside in normotensive dogs. Thus the effects of nitroprusside on blood flow to ischemic myocardium may vary, depending on the doses used and the levels of blood pressure attained.

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Because coronary steal may significantly limit the salutary hemodynamic effects of nitroprusside in acute myocardial infarction, we proposed in this study to evaluate the effects of nitroprusside on myocardial blood flow and infarct size in instrumented awake dogs. In addition, we compared nitroprusside with pinacidil, a new antihypertensive agent with potent coronary vasodilator action.7–9 Nitroprusside and pinacidil were administered intravenously to produce a 25 mm Hg decrease in mean arterial pressure. Like nitroprusside, pinacidil is a direct-acting vasodilator and does not exert a direct stimulatory action on the heart or the sympathetic nervous system.8 In addition, it is capable of increasing myocardial blood flow twofold.8 Since the extent of coronary steal is proportional to coronary vasodilation,4 we speculated that the marked vasodilator action of pinacidil would cause a greater coronary steal than nitroprusside.

Methods

Surgical preparation. Adult healthy beagles weighing 9.4 to 15.4 kg were used. Animals were given intravenous sodium pentobarbital (25 mg/kg) and placed on a Harvard respirator (Harvard Apparatus, S. Natick, MA). A sterile left thoracotomy was performed via the fifth intercostal space. With the heart exposed, the left anterior descending coronary artery was dissected free below the tip of the left atrial appendage, and a silicone rubber Jones’ balloon occluder (internal diameter 3.5 mm, R. E. Jones, Silver Springs, MD) was implanted around the vessel. The balloon was transiently inflated with saline to determine the amount of saline required to produce total occlusion. Tygon catheters (internal diameter 1.02 mm, Norton, Plastic and Synthetics Division, Akron, OH) were inserted into the left atrium via the appendage, the main pulmonary artery, and the descending thoracic aorta. Catheters were then tunneled subcutaneously, with the ends sealed and buried in a pouch at the nape of the neck. The wound was closed and the animals were allowed to recover.

Experimental procedure. Animals were studied 12 to 16 days after surgery. They were placed in a lateral decubitus position after sedation with subcutaneous morphine sulfate (0.5 mg/kg). The subcutaneous pouch was opened to expose the indwelling catheters, which were connected to Statham P23Db pressure transducers (Statham Instruments, Inc., Oxnard, CA) and a Brush 480 multichannel recorder (Gould, Inc., Instrument Systems Division, Cleveland) for measuring pressures. Heart rate was calculated from the electrocardiogram. With animals under local anesthesia with 0.5% lidocaine, a carotid artery was cannulated with a transducer-tipped Millar catheter (Millar Instruments, Inc., Houston), which was advanced into the left ventricular cavity with fluoroscopic guidance, and connected to the Brush recorder for measuring left ventricular pressure and the peak rate of pressure rise of left ventricular pressure (dp/dt). The ratio of left ventricular dp/dt at a developed pressure of 50 mm Hg during isovolumic contraction to the developed pressure (dp/dt/P) was obtained and used as a measure of left ventricular contractility independent of aortic pressure.10

Cardiac output was determined by indocyanine green (Cardio-Green; Hynson, Westcott and Dunning Co., Baltimore) injected into the pulmonary artery, and sampled from the aorta by a Gifford Model 140 cardiac output system (Gifford Instruments, Inc., Oberlin, OH). Total peripheral vascular resistance was calculated by the conventional formula. Organ blood flows were measured by the radioactive microsphere method.11 Radioactive microspheres (New England Nuclear, Boston), 15 ± 3 μm in diameter and labeled with cerium-141, tin-113, ruthenium-103, niobium-95, and scandium-46 at a specific activity of 10 μCi/g, were suspended in a 10% dextran solution containing 0.01% Tween 80 and adequately sonicated before use. Microspheres were injected into the left atrium followed by a 10 ml normal saline flush over 30 sec. Arterial reference blood was collected beginning 10 sec before injection of microspheres and continuing for 90 sec thereafter at a rate of 7.75 ml/min via a Harvard pump. Approximately 1 million microspheres were injected before coronary artery occlusion and after reperfusion, while 2 to 3 million microspheres were injected during the period of coronary occlusion.

After the animal was killed with lethal doses of sodium pentobarbital, the heart was removed immediately for determination of region at risk and infarct size.12,13 A cannula was inserted into the left anterior descending coronary artery at the site of the balloon occluder. Separate catheters were inserted into the left main and right coronary arteries via their respective ostia. The heart was then perfused for 15 min under a constant pressure of 100 mm Hg with a 1% Acramin Pink dye solution (Mobay Chemical Co., Rock Hill, SC) into the left anterior descending artery and a 0.5% monastral blue dye solution (Sigma Chemical Co., St. Louis) into the other two catheters. The area stained red was the “risk region.”12 After staining, the left ventricle, including the interventricular septum, was separated from the rest of the heart and cut transversely into six or seven slices approximately 6 to 7 mm thick. The tissue slices were then weighed, photographed, and immersed in a nitroblue tetrazolium solution for 15 min at 37°C. Nitroblue tetrazolium, a marker for tissue dehydrogenase,14 stains viable myocardium blue. The unstained area was considered infarcted. The tissue slices were then rephotographed.

Photographs were planimetered to determine the risk and infarct areas relative to the entire slice. Values for both the apical and basal sections of the slice were averaged and then multiplied by weight to obtain the risk area and infarct size for that slice. Total risk region and infarct size of the heart were determined by additions of individual values of all slices. Each slice then was cut radially into four to nine wedge-shaped pieces, each of which was divided into endocardial and epicardial halves, for a total of 84 to 100 segments. The segments were weighed and counted for radioactivity for measuring regional blood flows.

Left ventricular transmural segments were grouped into four regions according to their endocardial blood flows determined 40 min after coronary artery occlusion.13 Transmural segments with endocardial blood flow less than 25 ml/100 g/min were designated as severely ischemic. Segments with endocardial blood flows of 25 to 50 ml/100 g/min and 50 to 75 ml/100 g/min were designated as moderately and mildly ischemic, respectively. Areas with flows greater than 75 ml/100 g/min were considered nonischemic.

Experimental protocol. Experiments were begun at least 1 hr after carotid cannulation and 90 min after administration of morphine sulfate. Baseline hemodynamic values, including systemic hemodynamics and myocardial blood flows, were obtained during a 20 min control period. The balloon occluder previously implanted around the left anterior descending coronary artery was then inflated with the predetermined amount of normal saline to produce acute anterior myocardial infarction, which was evidenced by the characteristic ST segment elevation and immediate increases in heart rate and left atrial pressure. The coronary artery balloon occluder was deflated 4 hr later.

Animals were randomly assigned to receive an intravenous
infusion of normal saline, nitroprusside, or pinacidil beginning 40 min after the onset of coronary artery occlusion. The doses of nitroprusside and pinacidil were titrated to produce a 25 mm Hg decrease in aortic pressure. The target aortic pressure was achieved within 10 min, and the rate of infusion was kept unchanged thereafter. Infusions continued throughout coronary artery occlusion and the first 1 hr of reperfusion. The total volume infused was measured by weighing the solution bag before and after the infusion. The volume of normal saline infused matched those of pinacidil and nitroprusside. Animals were returned to the vivarium after the experiment and killed with lethal doses of intravenous pentobarbital on the following day.

The experimental protocol was identical in the three groups of dogs, except for the differences in their drug assignments. Systemic hemodynamics were obtained at 5 min intervals during the preocclusion baseline period and at 5 to 10 min intervals during the first hour of coronary artery occlusion. Subsequently, systemic hemodynamics were measured in duplicate at 2, 3, and 4 hr of coronary artery occlusion and at 15, 30, and 60 min after reperfusion. Regional blood flows were determined during the control period, at 40 min, 1 hr, and 4 hr of coronary artery occlusion, and 1 hr after reperfusion.

Statistical analysis. The experimental results are given in mean ± SE. The statistical significance of the difference between the three experimental groups was determined by two-way analysis of variance for repeated measures in independent groups.15 Dunnett’s test16 was used to determine the significance of differences between the control value and the serial repeated measurements after coronary artery occlusion and during reperfusion in each group. Student’s t test was used to analyze the statistical significance of a difference between two means. Differences were considered statistically significant if p < .05.

Results

Twenty-nine dogs with acute myocardial infarction after coronary artery occlusion received normal saline (n = 9, 10.5 ± 0.3 kg), nitroprusside (n = 10, 11.0 ± 0.7 kg), or pinacidil (n = 10, 10.6 ± 0.5 kg). To achieve the desired decrease in mean aortic pressure, nitroprusside and pinacidil were infused at 5.2 ± 0.6 and 4.0 ± 0.6 µg/kg/min, respectively. All animals survived the short-term hemodynamic study, but six dogs (two in each group) died overnight; only 23 dogs were available for postmortem determination of infarct size.

Systemic hemodynamics. Effects of coronary artery occlusion and drug infusions on mean aortic pressure and other variables of the systemic hemodynamics are shown in figures 1 and 2, respectively. In the 29 dogs studied, acute coronary artery occlusion increased heart rate (from 98 ± 3 to 114 ± 4 beats/min; t = 5.24, p < .001) and left atrial pressure (8.0 ± 0.5 to 10.3 ± 0.6 mm Hg; t = 5.78, p < .001) but did not change mean aortic pressure, cardiac output, left ventricular dP/dt and dP/dt/P before initiation of drug infusions.

As expected, mean aortic pressure decreased during infusion of nitroprusside and pinacidil (figure 1). The magnitude of the decrease in aortic pressure was similar between the two groups. Mean aortic pressure did not change during infusion of normal saline.

Pinacidil increased cardiac output, heart rate, and left ventricular dP/dt and dP/dt/P (figure 2). These changes persisted throughout coronary artery occlusion and the reperfusion period. In contrast, neither nitroprusside nor normal saline increased cardiac output, heart rate, or left ventricular dP/dt and dP/dt/P. Heart rate actually decreased to preocclusion values between 2 and 3 hr of coronary artery occlusion during infusion of nitroprusside.

Left atrial pressure decreased during nitroprusside and pinacidil infusions. Nitroprusside decreased left atrial pressure from 12 ± 1 to 6 ± 1 mm Hg (t = 8.73, p < .001) within 20 min of infusion. With pinacidil infusion, left atrial pressure decreased from 10 ± 1 to 7 ± 1 (t = 4.31, p < .001) within 20 min. Left atrial pressure remained decreased throughout the occlusion and reperfusion phases in the nitroprusside and pinacidil groups. At the end of the 4 hr occlusion, left atrial pressure was 7 ± 1 mm Hg in both of these groups. In contrast, infusion of normal saline did not decrease left atrial pressure, which was 10 ± 1 mm Hg at the end of the experiments.

Regional myocardial blood flows. The relationship of myocardial blood flow and anatomic location of each transmural piece was examined. The severely ischemic regions were within the distribution of left anterior descending coronary artery and surrounded by mildly and moderately ischemic regions.

Figure 3 shows that normal saline had no effect on myocardial blood flow to the nonischemic region.

![FIGURE 1. Changes in mean aortic pressure after coronary artery occlusion and reperfusion in three experimental groups. Animals received infusions of normal saline, nitroprusside, or pinacidil beginning 40 min after the onset of coronary artery occlusion. Bars denote SE. Asterisks indicate values that differ from the preinfusion values at p < .05.](http://circ.ahajournals.org/content/77/3/707)
Nitroprusside also did not change myocardial blood flow within 20 min of infusion but significantly increased epicardial blood flow at 4 hr of coronary artery occlusion. It also increased endocardial and epicardial blood flows during reperfusion. The increase in nonischemic myocardial blood flow produced by nitroprusside was modest, averaging 20% to 40% of the baseline values. In contrast, pinacidil produced three- to fourfold increases in endocardial and epicardial blood flows in the nonischemic region. The changes occurred within 20 min of infusion and persisted for the entire duration of the pinacidil infusion.

Myocardial blood flow decreased markedly after coronary artery occlusion in the most severely ischemic region (figure 4). Myocardial blood flow did not change further in this region during coronary artery occlusion in the normal saline group but was increased significantly by nitroprusside. On the other hand, pinacidil infusion did not increase blood flow to the ischemic region. With reperfusion, myocardial blood flow returned to the preocclusion values in the normal saline group, but increased to values that were significantly higher than the preocclusion control in the nitroprusside group. Myocardial blood flow also increased after reperfusion in animals receiving pinacidil, but unlike the other two groups, the increase in endocardial blood flow was disproportionately smaller than that in epicardial blood flow.

The relationships between endocardial and epicardial blood flows were studied by endocardial/epicardial blood flow ratios. Figure 5 shows that the endocardial/epicardial flow ratio in the severely ischemic region decreased markedly after coronary artery occlusion. This ratio was increased by nitroprusside, suggesting a proportionately greater increase in endocardial flow than epicardial flow. The endocardial/epicardial flow ratio was changed by neither normal saline nor pinacidil during coronary artery occlusion. With reperfusion, the endocardial/epicardial ratio returned toward unity but was still below the preocclusion values in the normal saline and nitroprusside groups. In contrast, the endocardial/epicardial ratio did not increase with reperfusion in the pinacidil group.

**Myocardial infarct size.** Table 1 shows the risk regions and infarct sizes in the normal saline, nitroprusside, and pinacidil groups. Body weight and left ventricular weight did not differ among the three groups. The three experimental groups also had similar normalized risk regions, but the nitroprusside group showed a significantly smaller infarct size than the two other groups. Infarct size did not differ significantly between the normal saline and pinacidil groups.
Discussion

The present study shows that nitroprusside and pinacidil exert differential effects on ischemic myocardium. At equipotent hypotensive doses, only nitroprusside increased blood flow to the ischemic myocardium and the endocardial/epicardial blood flow ratio. Moreover, only nitroprusside reduced infarct size. Infarct size was affected by neither pinacidil nor normal saline.

Nitroprusside has been shown to have short-term beneficial effects on global left ventricular function and on function of regional ischemic myocardium during acute myocardial infarction. These changes probably are related to the decreases in preload and afterload. However, whether this agent reduces ischemic injury or infarct size remains controversial. Early studies have shown that nitroprusside reduces precordial ST segment elevation and reduces mortality in patients with acute myocardial infarction. Contradi-
tory reports, however, indicate that nitroprusside may reduce blood flow to the ischemic myocardium and increase epicardial ST segment elevation in experimental animals. Other studies have shown no late effect of nitroprusside on mortality and morbidity in patients after acute myocardial infarction. The reasons for these discrepant results have not been fully explained but probably were related, at least in part, to the differences in the doses of the drug, the levels of blood pressure reduction attained, the presence or absence of ventricular dysfunction, and the timing of its administration in relation to the onset of acute myocardial infarction.

Nitroprusside exerts varying effects on ischemic myocardial blood flow. Results of our prior study in hypertensive dogs suggest that the effects of nitroprusside on ischemic myocardium are influenced by the doses of nitroprusside and the degree of blood pressure reduction. As shown previously in the hypertensive dogs, nitroprusside, which produced a 25 mm Hg reduction in mean aortic pressure, increased blood flow to the ischemic myocardium in the present study. This change in blood flow probably was caused by the vasodilator effect of nitroprusside on coronary collateral

FIGURE 3. Changes in blood flow to nonischemic myocardium after coronary artery occlusion and reperfusion in three experimental groups. Bars denote SE. Asterisks indicate values that differ from preocclusion controls at p < .05. Daggers indicate values that differ from preinfusion values obtained at 40 min after the onset of coronary artery occlusion at p < .05.

FIGURE 4. Changes in blood flow to ischemic myocardium after coronary artery occlusion and reperfusion in three experimental groups. See figure 3 for explanations of symbols.
vessels. Both nitroprusside and nitroglycerin have been shown to increase coronary collateral flows. However, ischemic myocardial blood flow has been shown to decrease as blood pressure falls below a critical level during infusion of nitroglycerin or nitroprusside; this change is reversed when methohexamine is used to restore the blood pressure to control values. Compared with nitroglycerin, at equipotent doses nitroprusside is more prone to decrease blood flow to ischemic myocardium and exaggerate ischemic injury. It has been suggested that nitroprusside is more likely to cause coronary steal because it dilates small resistance vessels whereas nitroglycerin dilates large coronary arteries.

The reduction in infarct size by nitroprusside in our study probably was related to the increase in ischemic myocardial blood flow and a decrease in myocardial oxygen demand as nitroprusside decreased both the preload and afterload of the heart. The reduction in preload probably also plays a role in the improvement in endocardial blood flow.

In contrast to nitroprusside, pinacidil had no effect on ischemic myocardial blood flow and infarct size in our present study, despite similar reductions in mean aortic pressure in the two groups. Also unlike nitroprusside, pinacidil caused a significant increase in heart rate, cardiac output, and left ventricular dP/dt and dP/dt/P. Earlier studies (Cohen and Colbert, Pinacidil monohydrate, Clinical Investigation Manual. 1985, Eli Lilly and Co., p 17) have shown that pinacidil has no direct positive inotropic effects in isolated atria. The positive chronotropic and inotropic effects of pinacidil in intact animals most likely are caused by baroreceptor-mediated sympathetic stimulation because these effects are abolished by β-adrenoceptor blockade.

Although pinacidil did not increase collateral flow to the ischemic myocardium, it increased coronary blood flow to nonischemic myocardium. The coronary dilator action of pinacidil cannot be explained entirely by the increased myocardial oxygen consumption caused by the chronotropic and inotropic effects of pinacidil, nor is it mediated via prostaglandin synthesis or adenosine receptors. Recent studies suggest that pinacidil may exert its vasodilator effect via a novel mechanism in that it enhances potassium conductance across the vascular smooth muscle cell membrane, resulting in hyperpolarization. In the ischemic region, pinacidil did not increase myocardial blood flow or the endocardial/epicardial flow ratio during coronary artery occlusion. The persistent reduction in endocardial/epicardial flow ratio after reperfusion indicates that pinacidil preferentially increased blood flow to the epicardial region.

Nitroprusside did not increase cardiac output, probably because nitroprusside also dilates capacitance vessels and reduces venous return. In addition, infusion of nitroprusside seems to be associated with less marked reflex stimulation of the heart than pinacidil, since neither heart rate nor left ventricular dP/dt/P increased during infusion of pinacidil in this study. The findings are consistent with those of our earlier study in hypertensive dogs, in which nitroprusside did not increase heart rate, cardiac output, and left ventricular dP/dt and dP/dt/P at a dose that decreased aortic pressure by a magnitude similar to that of the present experiment. However, when aortic pressure was reduced further with larger doses of nitroprusside, significant chronotropic and inotropic changes were observed.

In summary, our study shows that at the doses administered, nitroprusside is an efficacious agent during acute myocardial infarction and reperfusion. It improves blood flow to the ischemic myocardium and reduces infarct size. In contrast, at equipotent hypotensive doses, pinacidil neither increases ischemic myocardial blood flow nor reduces infarct size.

![Graph](image.png)

**FIGURE 5.** Changes in the endocardial/epicardial blood flow ratio after coronary artery occlusion and reperfusion in three experimental groups. See figure 3 for explanations of symbols.

**TABLE 1**

<table>
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<tr>
<th>Effects of nitroprusside and pinacidil on infarct size (mean ± SE)</th>
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<tr>
<td><strong>No. of experiments</strong></td>
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<tr>
<td><strong>Body weight (kg)</strong></td>
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<tr>
<td><strong>LV weight/body weight (g/kg)</strong></td>
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<td><strong>Risk region (% LV weight)</strong></td>
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LV = left ventricular.

*p < .05 vs saline; †p < .05 vs nitroprusside.
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References

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