The Effects of Intravenous Nitroglycerin on Hemodynamics, Coronary Blood Flow and Morphologically and Enzymatically Estimated Infarct Size in Conscious Dogs

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SUMMARY Nitroglycerin (TNG) decreases ST-segment elevation accompanying myocardial ischemia, but its effect on morphometrically and enzymatically estimated infarct size (IS) has not been defined. Accordingly, coronary occlusion was produced in 92 conscious dogs; 65 survived for 24 hours. Thirty-three received TNG (200–300 μg/min i.v. for 8 hours) and the results were compared with those in 32 untreated dogs. Coronary blood flow (CBF) was measured with tracer microspheres (113Ce, 85Sr and 82Sr) 5 minutes after occlusion before TNG, 20 minutes after TNG and again at 8 hours. Mean blood pressure decreased from 103 to 84 mm Hg with TNG, vs 99 to 94 mm Hg in controls (p<0.02). Nitroglycerin increased CBF in the subendocardium of ischemic areas by 45% (0.09 to 0.13 ml/min/g). The dogs were sacrificed after 24 hours and IS was estimated morphometrically (25 ± 1% vs 27 ± 1% of left ventricular weight) and from myocardial CK depletion (23 ± 1% vs 24 ± 1%) were similar for the two groups. Thus, despite increased subendocardial CBF, prolonged i.v. TNG did not decrease infarct size, although a 15% difference would have been detected with this sample size. TNG may relieve coronary spasm but does not appear to be beneficial with sustained coronary occlusion.

NITROGLYCERIN has long been used clinically for the treatment of angina pectoris, although the basis of relief of angina is controversial. Nitroglycerin reduces ventricular afterload and preload by venular and arteriolar dilatation and has been proposed as the predominant mechanisms for relief of angina. Whether nitroglycerin increases coronary flow as a result of direct coronary vasodilatation is controversial, but redistribution of flow to the ischemic subendocardium is well documented and may play a role in its beneficial effect. Recently, nitroglycerin was found to be beneficial in patients with heart failure that occurred in association with acute myocardial infarction. The recent use and availability of i.v. nitroglycerin and the demonstration of a beneficial effect on myocardial ischemia in experimental animals have encouraged its use in patients with acute myocardial infarction, despite the possible reduction of coronary perfusion pressure.

Smith et al. showed that nitroglycerin administered intravenously to dogs with evolving myocardial infarction was associated with decreased ST-segment elevation. Flaherty et al. observed similar effects in patients with acute myocardial infarction. When methoxamine or phenylephrine was administered with nitroglycerin to prevent the associated drop in blood...
pressure, which may occur when nitroglycerin is administered alone, the beneficial effect of nitroglycerin on ischemic myocardium was further enhanced.\textsuperscript{6, 11, 12} However, Come et al.\textsuperscript{13} observed that the beneficial effect of nitroglycerin is abolished when it is combined with phenylephrine. Whether nitroglycerin in experimental animals or in patients is beneficial in uncomplicated acute myocardial infarction is not known. Apparent beneficial effects of nitroglycerin on myocardial ischemia during myocardial infarction have been detected, based on reduction of associated ST-segment elevation. ST-segment elevation may reflect ischemia with or without necrosis, so it remains to be determined whether nitroglycerin reduces infarct size as determined morphologically or enzymatically. Furthermore, in most studies, nitroglycerin was administered for only brief intervals and usually to anesthetized or heavily sedated animals. Thus, the present study was performed in unsedated, conscious dogs with myocardial infarction without hemodynamic failure to determine the effect of prolonged administration of nitroglycerin on hemodynamics, coronary blood flow (determined by the microsphere technique) and infarct size estimated morphologically and enzymatically. Results in dogs treated with i.v. nitroglycerin for 8 hours were compared with results in control dogs.

**Materials and Methods**

Studies were performed in 92 unsedated, conscious, male mongrel dogs that weighed 14–30 kg. Myocardial infarction was produced by occlusion of the left anterior descending coronary artery. Anesthesia was induced with thiopental (15 mg/kg), and sustained by halothane (0.5%) and ventilation was maintained by a Harvard pump. A left thoracotomy was performed via an incision in the fourth intercostal space. The pericardial sac was opened and a suture placed loosely around the left anterior descending coronary artery immediately proximal to the first major branch, which is about 2 cm from its origin in small dogs and 4 cm in large dogs, to form a snare. Both ends of the suture were inserted into a polyethylene catheter, with the distal end attached to the myocardium and the proximal end exteriorized and secured to the skin as previously described.\textsuperscript{14} Silastic catheters were placed into the jugular vein and carotid artery for blood sampling and monitoring of hemodynamics.

To measure left atrial pressure, a catheter was placed in the left atrium and exteriorized to the skin. The catheter was also used to inject microspheres to determine regional myocardial blood flow. A catheter was inserted into the femoral artery to obtain reference blood samples to calculate blood flow. All catheters were flushed daily with heparin to maintain patency. Systemic arterial and left atrial pressures were recorded on a Gould brush direct-writeout recorder via the catheters in the carotid artery and left atrium and the heart rate and rhythm were monitored by a single ECG lead (lead 1). We have shown\textsuperscript{15} that by occluding at approximately the same site on the left anterior descending coronary artery and normalizing for the variation in heart size by expressing infarct size as a percentage of left ventricular weight, the variation in infarct size is reduced. Infarct size based on CK depletion ranged from 13,200–71,000 units (mean ± SD 39,623 ± 19,231 units). When expressed as a percent of heart weight, the mean was 25.2 ± 7.8%. However, to assess whether nitroglycerin affects the relationship between morphologic and enzymatic estimates of infarct size over a wider range of infarct size than afforded by the proximal occlusions, snares were placed on the distal portion of the left anterior descending coronary artery in eight additional dogs (four controls and four treated) and results were analyzed for this purpose only and were not included in the results analyzed to determine the effect of nitroglycerin on infarct size.

Five to 10 days later, when the dogs had recovered completely from surgery and plasma CK activity had returned to normal, coronary occlusion was produced by tightening the previously exteriorized snare.\textsuperscript{14} Blood samples were obtained before occlusion and every 2 hours thereafter for 24 hours for CK determinations. Samples were collected in EGTA (0.005 M) and plasma was separated from cellular components by centrifugation (2000 g for 10 minutes) and stored in mercaptoethanol (0.005 M). Enzyme analysis were performed on all samples within 3–6 weeks of storage, an interval shown to be associated with no loss of enzyme activity.\textsuperscript{16}

**Administration of Nitroglycerin**

Nitroglycerin solutions were prepared immediately before each experiment by dissolving sublingual tablets (0.6 mg) in sterile saline in a final concentration of 1.2 mg/ml. Before coronary occlusion, dogs were allocated on an alternate basis to control and treated groups. The control dogs received an i.v. infusion of sterile saline. In the dogs that received nitroglycerin, an 8-hour infusion was initiated within 10 minutes of coronary occlusion at a dose of 300 µg/min but adjusted so that systolic pressure did not drop below 90 mm Hg. The dose required ranged from 200–300 µg/min. This dose was chosen on the basis of previous studies that showed that the minimum dose to exhibit a cardiovascular effect in the conscious dog was 160 µg/min,\textsuperscript{17} and studies that showed a beneficial effect of nitroglycerin on ST-segment elevation in the dog were performed with doses of 200–400 µg/min.\textsuperscript{9} However, to determine whether lower doses may be associated with greater increases in coronary flow, seven dogs received an infusion of only 100 µg/min.

**Determination of Myocardial Blood Flow**

In 29 dogs (14 treated and 15 controls), myocardial blood flow was measured with radioactive microspheres, 7–9 µm in diameter, labeled with 141Ce, 85Sr and 99Nb (3M Company). The microspheres were suspended in 10% dextan and before each injection were agitated vigorously in an ultrasonicator for 5 minutes and mixed thoroughly with a magnetic stirrer for at
least 15 minutes. To be sure the microspheres had not
aggregated, a sample of the injectate was examined
microscopically before injection. Bolus doses contain-
ing approximately $4 \times 10^9$ microspheres were injected
into the left atrium over 10-second intervals and
flushed with saline (3 ml). A reference arterial blood
close was collected from the catheter in the femoral
artery 10 seconds before each microsphere injection
and was continued for 90 seconds at a constant speed
of 7.6 ml/min using the Harvard pump (model 940).

To determine regional myocardial blood flow at
selected intervals after coronary occlusion, a micro-
sphere suspension with different radioisotopes was in-
jected 5 minutes after coronary occlusion (which, in
the treated group, was 5 minutes before initiation of
nitroglycerin therapy), 20 minutes after onset of
nitroglycerin, and 8 hours after onset of nitroglycerin
therapy. The sequence of injections of radionuclides
was randomized. Comparable injections of radioac-
tively labeled microspheres during comparable inter-
vals after coronary occlusion were performed in the
controls. Twenty-four hours after coronary occlusion
the dogs were sacrificed and biopsies were obtained (4
g/biopsy) from the center of the area of infarction,
surrounding peripheral zone, and normal tissue (0.5 g)
from the posterior ventricular wall. Each biopsy was
divided into epicardial and endocardial layers to de-
termine the transmural distribution of myocardial flow.

To determine whether dropout of microspheres oc-
curs in ischemic areas, radioactively labeled microspheres were injected in four dogs before cor-

onary occlusion, 5 minutes after and 8 hours after cor-

onary occlusion and the dogs were sacrificed 24 hours
after occlusion. Coronary flow was not determined
before occlusion in the other 25 dogs, because it was not
necessary to determine the effect of nitroglycerin on
infect size or coronary flow and we wanted to
minimize the problem of energy overlap of the
microspheres and keep the number of microspheres per
dog to a minimum. Samples were analyzed in a
well-type gamma counter for radioactivity, after
which the samples were homogenized and assayed
spectrophotometrically\textsuperscript{15, 18} for CK activity. The loca-
tion of the biopsy was confirmed from the myocardial
CK activity present, i.e., whether it was from a nor-
mal, peripheral or central area of infarction. Regional
myocardial blood flow was calculated by comparing
radioactivity per gram to that obtained in the blood as
previously outlined.\textsuperscript{5, 19, 20}

**Morphologic Assessment of Infarct Size**

Twenty-four hours after coronary occlusion the
dogs were anesthetized with sodium pentobarbital
and the heart was rapidly excised and washed three
times in cold saline. The left ventricle was sliced care-
fully into cross-sectional full-thickness sections (six to eight
slides) from apex to base, and the area of infarction was
detected by gross inspection, removed, weighed and
expressed as a percentage of the total weight of the
left ventricle. Borders were not defined by staining
techniques because of possible nonspecificity of
available stains for cell necrosis and their interference
with spectrophotometric determination of CK enzyme
activity. Enzymatic estimates preclude simultaneous
determinations of histologic assessment in the same
hearts. As absolute values, our gross morphologic es-

timates of infarct size performed after 24 hours may not be as accurate as estimates after 72 hours or those
assessed by refined histologic techniques. However,
despite these limitations, the morphologic approach
was identical in treated dogs and controls, so we feel
the estimates are valid for comparative purposes and
are valuable as adjuncts to the enzymatic approach.

**Enzymatic Estimation of Infarct Size**

In the present study, our emphasis was on the deter-
mination of infarct size based on myocardial CK
depletion and morphology rather than that of CK
released into the blood, because nitroglycerin in-
creases coronary flow and may alter the ratio of CK
released into the blood compared with that lost from
the heart, thereby affecting plasma CK curves.

However, infarct size determined based on measure-
ment of myocardial CK content is independent of fac-
tors that may affect plasma CK activity, the dis-
appearance rate or distribution volume.\textsuperscript{18} CK activity
in blood samples, biopsies and in the homogenates of
myocardium were assayed spectrophotometrically as
previously described.\textsuperscript{18} Biopsies were homogenized in
Tris-HCl (10 mM), BSA (0.002%) and mercap-
toethanol (0.001 M) at a pH of 7.4.\textsuperscript{18} The normal
myocardial CK activity was determined for each heart
from four transmural biopsies (0.5 g/biopsy) obtained
from the nonischemic normal posterior wall and
results were averaged to estimate normal CK activity
per gram for that heart. The mean CK activity per
gram of normal myocardium determined for each
heart multiplied by the total weight of the left ventricle
represented the expected total myocardial CK activity
before coronary occlusion in that particular heart.
The whole left ventricle was then homogenized together
with the biopsies and the total actual CK activity pre-

tent determined. Thus, the normal expected CK ac-

tivity minus the activity present represents the amount
of CK depleted from the myocardium as a result of
coronary occlusion. The total amount of CK activity
depleted from the myocardium expressed as a percent-
age of the total expected represents an index of infarct
size, normalized for variation in heart size observed
from dog to dog.\textsuperscript{18} Using this approach and a standard
site of occlusion of the coronary artery (2–4 cm distal
to the origin), we have detected modest changes in in-
farct size in relatively small groups of animals.\textsuperscript{21} In-
farct size was also assessed from changes in plasma
CK activity as previously described.\textsuperscript{18}

In each dog biopsies were obtained from the center of
the infarction and the surrounding peripheral zones
for determination of CK activity and myocardial
blood flow. Also, to determine whether CK activity is
uniformly distributed throughout the right and left
ventricles, 18 biopsies were obtained from each of four
dogs (nine from each ventricle) who did not undergo
coronary occlusion. Four samples from the left ventricle of each of the four dogs were separated into endocardial, myocardial and epicardial layers to evaluate the transmural distribution of myocardial CK activity.

Statistical Analysis

To assess the effect of nitroglycerin on infarct size, the values for infarct size in the treated group were compared with those in the control group using the nonpaired t test. The power function of the t test was determined and based on our sample size and an α level of 0.05; an analysis was performed to determine the beta level that would be necessary to detect differences in infarct size. Regional myocardial blood flow and hemodynamics in the treated group were compared with those of the control group by non-paired t test. In addition, using the general linear models procedure of the statistical analysis system,23 an analysis of variance was performed to assess the effects of each variable after controlling for the effects of the other. Thus, the effect of time on blood pressure, left atrial pressure and heart rate was assessed after controlling for the effect of treatment and vice versa. The interactive effect of nitroglycerin and time on these hemodynamic variables was also assessed. A similar variance analysis that was more complicated in design was used to assess the independent and interactive effects of time, treatment, distribution (subendocardial and epicardial) and location (normal, peripheral and central ischemic zone) on regional myocardial blood flow. A paired t test was performed to determine whether regional myocardial blood flow or hemodynamics changed within each group.

We determined differences in infarct size between dogs that had a greater decrease in blood pressure as a result of nitroglycerin compared with those who had only a minor decrease in blood pressure; results of infarct size in 16 dogs that had more than a 20-mm Hg decrease in mean arterial pressure were compared (nonpaired t test) with those in the remaining 17 dogs in which the decrease in mean arterial pressure was less than 20 mm Hg. The 20-mm Hg cutoff was chosen because it represents the mean decrease compared with the mean control value in the treated group. The relationship of infarct size assessed from changes in plasma CK activity to infarct size assessed by morphology and CK depletion was assessed by linear regression analysis and the slopes were compared in the treated and control groups by analysis of variance.

Results

Hemodynamic Changes

Coronary occlusion was performed in 92 dogs, but 27 (29%) died before completion of the study (14 controls and 13 treated). Nine dogs in the control group and eight in the treated group died within 30 minutes of coronary occlusion. The remaining 10 died 8–22 hours after coronary occlusion. Infarct size was not analyzed in these dogs because it might not be comparable to that obtained after 24 hours. Furthermore, because the mortality rate was similar in treated and control dogs, it does not affect the conclusion regarding the effect of treatment. Bishop et al.28 reported a 27% mortality in conscious dogs and Hirshfeld et al.12 reported 29%, similar to our mortality rate. Rasmussen et al.24 reported a mortality rate of 52%.

Complete coronary occlusion was confirmed at autopsy in all dogs. The mean heart rates, arterial blood pressures and left atrial pressures in treated and control groups are summarized in figure 1. Mean heart rate before occlusion in the treated group was 96 ± 5 beats/min (SEM), similar to values in the control group (97 ± 4 beats/min). The heart rate increased significantly after coronary occlusion in both groups and reached a maximum at 8 hours of 170

![Figure 1. Hemodynamic changes in control and treated groups. The mean values for the control group are indicated by the open circles and that of the treated by the solid circles. Vertical bars represent the standard error. The mean heart rates in the treated and control groups were not significantly different. However, the mean arterial pressure during the interval of nitroglycerin in the treated group was 84 mm Hg, vs 94 mm Hg in the control group (p < 0.02), but were similar after discontinuation of the nitroglycerin. The left atrial (LA) pressure was 4.3 mm Hg during nitroglycerin and 6.0 mm Hg in the control group during the comparable interval.](http://circ.ahajournals.org/content/62/6/1230)
beats/min. However, the increase in heart rate after coronary occlusion was similar in both groups throughout the 24 hours of observation and showed no statistically significant difference. Ventricular premature complexes and ventricular tachycardia occurred in both groups beginning 3–4 hours after coronary occlusion and continued intermittently throughout the 24-hour period. Four hours after coronary occlusion the frequency of ventricular premature complexes averaged 6 per minute in both treated and control groups, and by 6 hours had increased to 23 ± 8 per minute in the control and 26 ± 9 per minute in the treated groups and to 91 ± 12 in the control and 90 ± 11 in the treated groups by 8 hours after coronary occlusion. No statistically significant difference was observed in the frequency of ventricular arrhythmias at any time between the treated and control groups.

Mean arterial pressure before coronary occlusion was similar in the treated (103 mm Hg) and the control groups (99 mm Hg). After initiation of i.v. nitroglycerin the mean arterial pressure decreased in the treated group, averaging 84 mm Hg throughout the 8 hours of infusion, which was less than in the control groups, which averaged 94 mm Hg (p < 0.02) (fig. 1). The mean arterial pressure in the treated and control groups was similar 24 hours after occlusion and significantly lower than the control values before occlusion (p < 0.001). Mean arterial pressure in the dogs that received nitroglycerin was 20 mm Hg less than the mean control value and approximately 10 mm Hg less than that observed in the control group. The decreased blood pressure with nitroglycerin was mainly the result of a decrease in systolic blood pressure, with only minor changes in diastolic pressure (average 7 mm Hg). Despite the decrease in systolic blood pressure, no compensatory increase in heart rate occurred; presumably, this was compensated for by the beneficial effect of reduced afterload. The mean left atrial pressure was similar in both groups before occlusion, averaging 2.4 mm Hg, but increased to 6.0 mm Hg in the control group (p < 0.01) and to 4.3 mm Hg in the treated group (p < 0.05).

Analysis of variance assessing the effects of time and nitroglycerin on heart rate, blood pressure and left atrial pressure in the treated and controls confirm these results with the t test. After controlling for the effect of time, the treatment had a highly significant effect on left atrial pressure (p = 0.0002) and blood pressure (p = 0.0002), but no effect on heart rate (p = 0.1079). Thus, nitroglycerin had no effect on heart rate compared to that of the control group. However, because of a possible difference in heart rate during the first 3 hours (fig. 1), this period was also analyzed separately; however, no statistical difference was observed (p = 0.0764). The effect of time and the interactive effect of time and treatment were also studied. After controlling for the effect of treatment, the effect of time on heart rate, blood pressure and left atrial pressure were significant in each case (p = 0.0001).

**Regional Myocardial Blood flow**

The values for regional myocardial blood flow in the control and treated groups are summarized in table 1. The ratios of endocardial to epicardial blood flow in the normal areas and the peripheral and central areas of infarction are summarized in figure 2. Total transmural flow in the normal areas 5 minutes after coronary occlusion averaged 0.91 ± 0.05 ml/min/g (SEM) in controls and 0.95 ± 0.07 ml/min/g in treated dogs. Coronary flow did not change significantly in the treated group 30 minutes or 8 hours after coronary occlusion compared with the value 5 minutes after occlusion but a significant increase did occur in the control group 8 hours after occlusion compared with

<table>
<thead>
<tr>
<th>Table 1. Myocardial Blood Flow Changes in Control and Nitroglycerin-treated Groups (ml/min/g)</th>
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<tbody>
<tr>
<td><strong>Endocardial layer</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Central ischemic zone</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>±0.02</td>
</tr>
<tr>
<td>Treated</td>
</tr>
<tr>
<td>±0.02</td>
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<tr>
<td>Peripheral ischemic zone</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>±0.03</td>
</tr>
<tr>
<td>Treated</td>
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<tr>
<td>±0.04</td>
</tr>
<tr>
<td>Normal zone</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>±0.06</td>
</tr>
<tr>
<td>Treated</td>
</tr>
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<td>±0.08</td>
</tr>
</tbody>
</table>

**Values are mean ± SEM.**

Compared with 5-minute value by paired t test:
- *p < 0.05.
- †p < 0.01.
- ‡p < 0.001.

No significant difference between control and treated group.
that 5 minutes after occlusion ($p < 0.05$). Endocardial flow in the normal areas was greater than epicardial flow, with an endocardial-to-epicardial ratio of $1.13 \pm 0.04$ in control and $1.11 \pm 0.03$ in treated dogs 5 minutes after coronary occlusion. The ratios in the normal areas remained essentially the same in both groups throughout the interval.

The peripheral zones in the controls had a total transmural flow of $0.35 \pm 0.04$ ml/min/g, approximately a 60% reduction from normal and corresponded to a 48% decrease in myocardial CK activity (table 2). The endocardial-to-epicardial ratio was reversed from that observed in the normal areas (average $0.63 \pm 0.04$ (fig. 2). Total flow in the controls increased significantly by 8 hours, to $0.50 \pm 0.05$ ($p < 0.001$); however, the endocardial-to-epicardial ratio remained unchanged. Flow in the peripheral zone in the treated dogs was similar to flow in controls 5 minutes after occlusion, with an endocardial-to-epicardial ratio of $0.58 \pm 0.05$. The 53% reduction in myocardial CK activity in this region was also similar to that of controls. However, the treated group, unlike the control group, had no significant increase in total coronary flow at 30 minutes or 8 hours after coronary occlusion.

Total transmural flow in the central area of infarction was markedly reduced in both treated and control dogs to $0.17 \pm 0.03$ and $0.18 \pm 0.04$ ml/min/g. The corresponding myocardial CK activity was also markedly reduced to only 33% (transmural) of normal in the control and 36% of normal in the treated dogs. Total flow did not change significantly with time (30 minutes and 8 hours) in either the control or treated group. Epicardial flow was much greater than endocardial flow in both treated and control dogs, with ratios of 0.31 and 0.37 5 minutes after occlusion. The endocardial-to-epicardial ratio in the controls decreased slightly 30 minutes after occlusion and further decreased 8 hours after occlusion. In contrast, in the treated group the endocardial-to-epicardial ratio increased significantly 30 minutes after occlusion ($p < 0.01$). However, 8 hours after occlusion the ratio, compared with that at 5 minutes after occlusion, was no longer significantly different. Despite the increase in endocardial flow 30 minutes after occlusion, it was still only 0.13 ml/min/g, approximately 15% of normal.

Analysis of variance of regional myocardial blood flow gives similar results to those obtained with the $t$ test. Analyses were performed to determine the effects of treatment, time, distribution of blood flow (endocardial vs epicardial) and location (normal, peripheral and central zones). The effect of time on regional myocardial blood flow is highly significant ($p = 0.0138$). Similarly, subendocardial and epicardial effects were significant ($p = 0.0001$) as was the location ($p = 0.0003$). The effect of nitroglycerin on regional myocardial blood flow compared with controls was not significant ($p = 0.0706$). In addition, there were significant interactive effects. The $p$ value for the interaction of time and treatment was 0.0353 and for distribution and location of blood flow, 0.001. The results of myocardial blood flow in the seven dogs that received a low dose of nitroglycerin (100 $\mu g$/min) were analyzed separately and are summarized in table 3. Mean aortic pressure before occlusion was 97 mm

![Figure 2](image_url)
Hg and decreased to 88 mm Hg during the nitroglycerin infusion and was not significantly different from that of the control group (p > 0.2). The results of blood flow both qualitatively and quantitatively are almost identical to those observed with the high dose of nitroglycerin, suggesting that on the basis of changes in coronary flow, beneficial effects would be similar to those of the high-dose group.

Microspheres in ischemic zones may, over time, be redistributed out of the ischemic zones.25, 26 Thus, experiments were performed to assess whether this occurred and to what extent during the interval involved in this study. In the four dogs (two control and two treated) in whom microspheres were injected before occlusion, biopsies 24 hours later from the normal and peripheral areas as well as the center of infarction were almost identical (0.81, 0.80 and 0.79 ml/min/g).

In both the treated and control dogs, the initial regional myocardial blood flow determined immediately after coronary occlusion before treatment in both the central and peripheral ischemic zones were identical in the treated and control groups. This suggests that nitroglycerin did not increase microsphere loss, because control values in the treated group would have been lower had greater loss occurred. In addition, values for regional myocardial flow were almost identical to those observed by others who have determined regional myocardial flow shortly after injection of microspheres.27, 28 Thus, significant loss of microspheres from the ischemic area did not appear to occur in the present study over 24 hours. Our results indicate that nitroglycerin did not elicit an increase in transmural myocardial flow in either the normal myocardium or in peripheral or central areas of infarction. Endocardial flow in the central area of infarction was significantly increased, but this difference was not significant at 8 hours.

Infarct Size

CK activity in the right ventricle of dogs without myocardial infarction averaged 1738 ± 203 IU/g (SD), and in the left ventricle, 1762 ± 187 IU/g. The transmural distribution of CK activity throughout the wall of the left ventricle was uniform (fig. 3). Mean left ventricular weight was 86.5 ± 4 g (SEM) in controls and 89.6 ± 3 g in treated dogs, indicating that the distribution of heart size was similar in the two groups. Infarct size determined morphologically averaged 25.4 ± 1.0% of the left ventricular weight in the control dogs, similar to values in the treated group (27.0 ± 1.3%) (fig. 4). Infarct size assessed morphologically in controls ranged from 13.7-37.5%, compared with 12.6-41.5% in the treated group. Given an α level of 0.05, a 15% reduction in infarct size would have been detected at a β level of 0.20 (i.e., power function of 0.80) with our sample size (32 treated and 32 controls). Thus, nitroglycerin did not significantly reduce infarct size determined morphologically. The power function of the test based on our sample size at an α level of 0.05 was performed to determine the β levels that would be necessary to detect various differences in infarct size (table 4).

Infarct size based on myocardial CK depletion in the controls ranged from 12,412-86,652 IU (average ± SD 35,527 ± 16,164). Normalized for heart weight, the mean infarct size was 23.6 ± 7.2%. In the treated group, myocardial CK depletion varied from 12,310-

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**Table 3. Myocardial Blood Flow Changes in Dogs That Received Low-dose Nitroglycerin (ml/min/g)**

<table>
<thead>
<tr>
<th></th>
<th>Endocardial layer</th>
<th>Epicardial layer</th>
<th>Transmural layer</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>30 min</td>
<td>8 hrs</td>
</tr>
<tr>
<td>Central</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ischemic zone</td>
<td>0.11</td>
<td>0.14*</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>±0.03</td>
<td>±0.04</td>
<td>±0.07</td>
</tr>
<tr>
<td>Peripheral</td>
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<td></td>
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</tr>
<tr>
<td>ischemic zone</td>
<td>0.31</td>
<td>0.31</td>
<td>0.38</td>
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<tr>
<td></td>
<td>±0.05</td>
<td>±0.05</td>
<td>±0.08</td>
</tr>
<tr>
<td>Normal</td>
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<tr>
<td>zone</td>
<td>1.08</td>
<td>0.97</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>±0.10</td>
<td>±0.07</td>
<td>±0.12</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
* p < 0.05 compared to 5 minute value by paired t test.
75,510 IU (average 37,562 ± 18,263). Normalizing for heart weight, infarct size averaged 23.3 ± 7.8%, almost identical to the controls (fig. 4). Given an α level of 0.05, a 20% reduction in infarct size would have been detected at a β level of 0.20 (i.e., power function of 0.80) with our sample size. The β levels for detection of various differences in infarct size determined enzymatically are summarized in table 4. Thus, nitroglycerin did not significantly reduce infarct size as determined by direct assessment of myocardial CK depletion. Total CK released into the blood determined from changes in plasma CK activity averaged 2544 ± 279 IU in the treated group and was not significantly different from that in controls (2311 ± 288 IU).

In dogs in which a significant decrease in blood pressure occurred, coronary perfusion could have been compromised to the extent that infarct size might have been increased. To assess this possibility, results of infarct size in 16 of the treated dogs in which blood pressure had decreased by more than 20 mm Hg were compared with those in the remaining 17 dogs in whom blood pressure decreased by less than 20 mm Hg. However, morphologic infarct size in the group with a blood pressure decrease of greater than 20 mm Hg was 27.2 ± 1.9% and almost identical to that in the group with a decrease less than 20 mm Hg, which averaged 26.8 ± 1.8%. Further, in the subgroup of low-dose (100 μg/min) nitroglycerin-treated dogs, infarct size determined morphologically (29.8 ± 2.8%) and by CK depletion (24.4 ± 2.5%) were not significantly different from that of high-dose (300 μg/min) nitroglycerin-treated dogs (26.3 ± 1.5% morphologically and 23.1 ± 1.7% by CK depletion) or control dogs (25.4 ± 1.0% morphologically and 23.6 ± 1.3% by CK depletion).

The relationship between infarct size determined by morphology and from myocardial CK depletion is illustrated in figure 5 for the control and treated groups. The correlation between the two methods in the treated group gives an r value of 0.86, compared with 0.86 in the control group. The correlation between infarct size determined by CK depletion and CK released into the plasma yielded an r value of 0.84 in the treated (fig. 6) and 0.78 in the control groups. The correlation between CK release and morphologic estimates in the treated group had r value of 0.78 and

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Comparison of infarct size in nitroglycerin-treated and control dogs. The bar graphs represent mean infarct size and the standard errors are indicated by the crossed bars. Infarct size by morphology and CK depletion are almost identical in the treated and control groups.

![Figure 5](http://circ.ahajournals.org/)

**Figure 5.** The correlation between infarct size by morphology and that of CK depletion in the control group and the treated group. The regression lines and correlation coefficients are $y = 0.93 x - 0.25$, $r = 0.86$ in control and $y = 0.84 x + 0.58$, $r = 0.86$ in treated group.

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**Table 4. Relationship Between Power Function and Detectable Differences in Infarct Size Determined Morphologically and Enzymatically**

<table>
<thead>
<tr>
<th>β level</th>
<th>Power function</th>
<th>Detectable difference (%) in infarct size for various power values</th>
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<td>13.7</td>
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<tr>
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<td>0.80</td>
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<table>
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<th>β level</th>
<th>Power function</th>
<th>Detectable difference (%) in infarct size for various power values</th>
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</tr>
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<td>0.90</td>
<td>23.8</td>
</tr>
</tbody>
</table>
0.79 in the controls (fig. 7). An analysis of variance as a test of the hypothesis of equal slopes between myocardial CK depletion and that of morphology and CK released into the plasma in the treated and control groups was performed, and no significant difference was found.

The CK activity per gram of myocardium determined in biopsies obtained from central zones of infarction averaged 23% (endocardial) of normal in the control and 24% (endocardial) in the treated group (table 2). These values are similar to those reported in previous studies.14, 18 The CK activity in the endocardial layer of infarction was consistently less than that observed in the epicardium, with a ratio of endocardial CK activity to epicardial CK activity of 0.45 ± 0.08 in the control and 0.40 ± 0.08 in the treated group. These results indicate that nitroglycerin did not change the relationship between morphologic and enzymatic determinations of infarct size. This might be anticipated in view of the lack of effect on total coronary flow. Thus, there was no difference between mean infarct size estimates based on morphology, myocardial CK depletion or plasma CK in control dogs compared with those treated with nitroglycerin.

![Figure 6](image1.png)

**Figure 6.** The correlation between CK depletion and CK released into the plasma in the control and treated groups. The regression lines and correlation coefficients are $y = 0.57x + 194$, $r = 0.78$ in control and $y = 0.69x - 59$, $r = 0.84$ in the treated group.

![Figure 7](image2.png)

**Figure 7.** The correlation between infarct size by morphology and CK released into the plasma in the control and treated group. The regression lines and correlation coefficients are $y = 118x - 189$, $r = 0.79$ in control and $y = 112x - 190$, $r = 0.78$ in the treated group.

**From others**, suggesting that evolution of infarction, uncomplicated by extension, is complete within 5–8 hours. Thus, by initiating nitroglycerin immediately after occlusion, the time for potential protection of ischemic myocardium was maximized. The effect of nitroglycerin was evaluated in conscious dogs to avoid potentially spurious effects of anesthesia on infarct size, reflex activity, coronary flow or hemodynamics and because studies in dogs in the conscious state are more analogous to patients in a clinical setting than to those in anesthetized dogs. Although we observed no significant increase in heart rate over that in controls, we found no beneficial effect on infarct size as determined by enzymatic or morphologic techniques. However, the results of any study assessing the effect of an intervention on infarct size should be interpreted with thorough consideration of the techniques used to assess end points.

Regional myocardial blood flow was assessed with the microsphere technique and showed that nitroglycerin exerted no effect on total coronary flow in either the normal or ischemic zones. The observation that nitroglycerin did not increase flow in the normal area is in keeping with observations of Michaelson et al.,32 Nakamura et al.,33 Chiarriello et al.,34 and Most et al.35 Becker27 observed a transient increase in nonischemic areas after bolus injections of nitroglycerin, but no change with continuous infusions in anesthetized animals. Bache28 observed a slight decrease in coronary flow with nitroglycerin in nonischemic areas in conscious dogs.

**Discussion**

In the present study nitroglycerin was administered intravenously for 8 hours to conscious dogs with acute myocardial infarction. The interval of 8 hours was chosen based on data from our laboratory14, 29 and from others30, 31 suggesting that evolution of infarction, uncomplicated by extension, is complete within 5–8 hours. Thus, by initiating nitroglycerin immediately after occlusion, the time for potential protection of ischemic myocardium was maximized. The effect of nitroglycerin was evaluated in conscious dogs to avoid potentially spurious effects of anesthesia on infarct size, reflex activity, coronary flow or hemodynamics and because studies in dogs in the conscious state are more analogous to patients in a clinical setting than to those in anesthetized dogs. Although we observed no significant increase in heart rate over that in controls, we found no beneficial effect on infarct size as determined by enzymatic or morphologic techniques. However, the results of any study assessing the effect of an intervention on infarct size should be interpreted with thorough consideration of the techniques used to assess end points.
We found no increase in total coronary flow to the ischemic areas. Leighninger et al.\textsuperscript{38} using the technique of retrograde flow, showed that collateral flow was increased 20–25% after administration of nitroglycerin. However, Kattus and Gregg\textsuperscript{30} could not confirm these studies and showed that collateral flow detected by the same technique did not change after administration of nitroglycerin. Mathes and Rival\textsuperscript{31} observed an increase in total collateral flow with a combination of nitroglycerin and adrenalin, but did not assess nitroglycerin alone. Weisse et al.\textsuperscript{34} found that with complete coronary occlusion via thrombus, there was no increase in collateral coronary flow using isosorbide dinitrate, but reported an increase if the artery was occluded with a catheter. Capurro et al.\textsuperscript{40} reported an increase in total coronary flow to the ischemic area with nitroglycerin, but this was in the anesthetized dog in which collaterals had previously developed after prolonged coronary artery constriction. Chiarieillo et al.\textsuperscript{34} observed an increase in total coronary flow to the ischemic area; however, nitroglycerin was administered after pretreatment with nitroprusside and the animals were anesthetized. Bache,\textsuperscript{28} studying the conscious dog, observed an increase in total coronary flow to the ischemic area, most of which was distributed to the endocardium. Fam and McGregor\textsuperscript{2} reported a decrease in total coronary collateral flow with nitroglycerin. Linder and Seeman\textsuperscript{41} also observed a decrease, as did Michaelson et al.\textsuperscript{29} Results of Pasyk et al.\textsuperscript{42} in the conscious dog with intravenous infusion or intracoronary infusion, found no change in total collateral flow to the ischemic area, similar to the results of Nakamura et al.\textsuperscript{33} Thus, in the two studies that were performed in conscious dogs, nitroglycerin in one case had no effect on total collateral flow\textsuperscript{42} and in the other study caused a slight increase.\textsuperscript{28} Whether the animal was anesthetized or conscious, only bolus injections of nitroglycerin were associated with a change in total coronary flow; continuous infusions of nitroglycerin without a previous bolus were not associated with any change in total coronary flow.\textsuperscript{27, 32, 33, 35} Assessment of flow in these studies was only for a few minutes after the bolus injection, so we could not determine the duration for which total coronary flow was increased.

However, in the treated dogs redistribution of flow occurred in the central zone of infarction, resulting in increased subendocardial flow. Nevertheless, by 8 hours after occlusion there was no statistical difference in coronary flow in the treated and control group. Subendocardial flow in the central zone of infarction averaged 0.09 ml/min/g 5 minutes after occlusion and increased to 0.13 30 minutes after occlusion. Despite this 43% increase, subendocardial flow was only 15% of normal. Redistribution of flow from the epicardium to the endocardium as a mechanism accounting for increased endocardial flow by nitroglycerin has been postulated.\textsuperscript{3, 5, 29, 39, 38, 45} The endocardial flow may also be the result of increased collateral flow independent of epicardial flow, or a decrease in ventricular wall tension. Our data did not provide insight into the mechanism for the increase in subendocardial flow in areas of ischemia. However, in the control dogs in the central areas of ischemia, epicardial flow increased from 0.27–0.36 ml/min/g, with no change in endocardial flow (0.10–0.12 ml/min/g. In contrast, in the treated group, epicardial flow did not change (0.27 vs 0.27 ml/min/g), but endocardial flow increased from 0.09–0.13 ml/min/g (p < 0.01). The lack of increase in epicardial flow in the treated group may have been caused by redistribution to the endocardium. Thus, our data tend to support the former hypothesis.

The possibility that we underestimated the effect of nitroglycerin on myocardial blood flow due to loss of microspheres from ischemic zones\textsuperscript{36, 26} is unlikely for several reasons. First, the experiments in which flow was determined before occlusion showed flow in the normal and ischemic zones to be identical, based on microspheres injected before occlusion. Had dropout of microspheres occurred in the ischemic area, the flow in these areas as determined by microspheres injected before occlusion would have been less than that in the normal zones. Second, the initial values for flow before treatment were identical to those of the control group, indicating that nitroglycerin did not enhance microsphere loss. Third, White et al.\textsuperscript{26} recently showed that loss of microspheres depends on sample size, number of microsphere injections and duration between occlusion and sacrifice of the animal. These authors observed that if the dogs are sacrificed within 2–4 days and the biopsies are at least 4 g and at least 6 $\times$ 10$^6$ microspheres were injected, there was no significant microsphere loss. In the present study we used 4-g biopsies and injected, on the average, 4 $\times$ 10$^6$ microspheres and sacrificed the dogs within 24 hours, so it is not surprising that no significant loss of microspheres occurred. Capurro et al.\textsuperscript{48} observed 10% loss of microspheres in the ischemic endocardium after 24 hours but no loss in the epicardium. The apparent loss of microspheres may be related to the sample size and the number of microspheres injected. Finally, because nitroglycerin did not enhance microsphere loss, as shown by the similar values for flow before treatment in both groups, it is important to emphasize that within the design of this study, even if microsphere loss did occur, it would have done so to a similar extent in both the treated and control dogs, and thus would not detract from our results as to the effect of nitroglycerin on coronary flow or infarct size compared with controls.

Administration of nitroglycerin\textsuperscript{8} may be associated with reflex tachycardia, which may offset its potential beneficial effects on ischemic myocardium. In the present study no significant increase in heart rate occurred in treated dogs compared with controls, probably because the blood pressure was maintained at 90–120 mm Hg. Most of the decline in pressure that did occur was systolic rather than diastolic. Reflex tachycardia observed with nitroglycerin is generally related more to a rapid drop in pressure than a gradual decline, as observed in this study. Smith et al.,\textsuperscript{9} who observed an increase in heart rate, administered a loading dose of 400 $\mu$g and an infusion dose of 300–400 $\mu$g/min, compared with the 200–300-
μg infusion dose used in this study without a loading dose.

Nitroglycerin is a potent dilator of venules and arterioles, and thus decreases ventricular preload as well as afterload. The reduction of preload in the present study was reflected by the lower left atrial pressure in the treated dogs, which averaged 4.3 mm Hg, compared with 6 mm Hg in the controls. However, heart rate did not increase, so cardiac output appeared to be well maintained. Further evidence that cardiac output was adequate includes the values for myocardial blood flow, which were almost identical in the treated and control dogs and were similar to values observed by other investigators. Dogs that received a lower dose of nitroglycerin had similar coronary flow.

In the present study, infarct size was measured based on morphology, myocardial CK depletion and plasma CK release. Although morphology is the conventional standard, its use in patients is complex because of concomitant previous damage, the temporal and spatial heterogeneity of new damage, and difficulties in early histologic delineation between dead and ischemic tissue. In the dog, complete occlusion is usually associated with transmural infarction and is morphologically less heterogeneous than damage in man. Infarct size varied considerably from animal to animal. The disparity was minimized somewhat by selection of a consistent site for occlusion and expression of results as a percentage of left ventricular weight, thereby normalizing in part for variation in heart size.

Myocardial CK depletion measured directly is an index of infarct size independent of CK released into the plasma. Its value correlated closely with morphology in rats, dogs, baboons and rabbits. The correlation is unaffected by a variety of interventions, including propranolol, hyaluronidase, nifedipine, nitroglycerin, verapamil, cobra venom and reperfusion. Myocardial CK depletion correlates also with attenuation of ultrasound, decreased myocardial 11C-palmitate uptake, electrocardiographic evidence of infarction and reduction in coronary flow detected by the microsphere technique. We have shown that myocardial CK depletion expressed as a percentage of left ventricular weight is reduced by halothane anesthesia and is increased by isoproterenol compared with values in controls, but is unaltered by late administration of verapamil. Despite our initial concern that nitroglycerin might alter the ratio of CK release into the plasma to that depleted, infarct size determined by CK release correlated closely with morphology and myocardial CK depletion. This is not surprising, because total coronary flow did not change significantly.

The lack of effect of nitroglycerin on infarct size as determined in this study may be expected, in view of the minor changes in coronary flow. Although coronary flow changed very little and although considerable attention has been focused on the redistribution of blood flow by nitroglycerin to the subendocardium, subendocardial flow was still only about 15% of normal. Also, nitroglycerin, unlike the calcium antagonists verapamil and nifedipine, increases subendocardial flow only in the central area of infarction. This is perhaps the area with least potential for salvaging ischemic myocardium.

Several investigators have reported a beneficial effect of nitroglycerin on myocardial ischemia as determined by changes in ST segments; however, overall infarct size was not determined morphometrically or enzymatically in these studies. The effects observed by these investigators of nitroglycerin may reflect beneficial effects on the severity of ischemia only. The effect of nitroglycerin on infarct size may have been minimal, being less than 15%, and thus would not have been detected in the present study. The purpose of the present study was to determine whether nitroglycerin alone altered uncomplicated myocardial infarction. Our results may not be comparable to those obtained under conditions in which nitroglycerin is combined with other agents such as α agonists or administered to animals with cardiac failure. Furthermore, in the present study there was an induced mean decrease in blood pressure, compared with controls, of about 10 mm Hg, which averaged 10%. Nitroglycerin administered in a smaller dose without significantly reducing peripheral vascular resistance or blood pressure may have a different result. The results of this study may not be applicable to all patients with myocardial infarction for another reason. Nitroglycerin dilates larger vessels and since spasm may play a role in some patients, relief by nitroglycerin may increase coronary flow substantially. Obviously, this type of potentially protective mechanism would not be detected in the model used in the present study because major vessels were permanently occluded.

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