Effect of Intravenous Nitroglycerin on Collateral Blood Flow and Infarct Size in the Conscious Dog

BODH I. JUGDUTT, M.B., LEWIS C. BECKER, M.D., GROVER M. HUTCHINS, M.D., BERNADINE H. BULKLEY, M.D., PHILIP R. REID, M.D., AND CLAYTON H. KALLMAN, Sc.M.

SUMMARY This study was performed to determine whether nitroglycerin (NG) can increase collateral flow to ischemic myocardium and reduce ultimate infarct size. Permanent occlusion of the mid-circumflex coronary artery was produced in 43 previously instrumented conscious dogs and within 3 minutes, 6-hour intravenous infusions were begun of saline (controls, n = 18), NG in doses to reduce mean arterial pressure by 10% but not below 90 mm Hg (n = 15), or NG followed by methoxamine (MX) to correct the NG-induced fall in blood pressure (n = 10). After sacrifice 2 days later, the occluded coronary bed was defined by postmortem coronary arteriography and masses of infarct and occluded bed were measured by planimetry of weighed rings of the left ventricle (LV). Infarct size was significantly less with NG than saline, both as a percent of LV (12.1 vs 6.4%, p < 0.05) and as a percent of occluded bed (32.0 vs 15.9%, p < 0.005). NG plus MX did not reduce infarct size more than NG alone: 6.6 vs 6.4% of LV, and 16.0 vs 15.9% of occluded bed. Masses of LV and occluded bed did not differ significantly among the three groups. Coronary blood flow (CBF), measured by 7-10-μm radioactive microspheres, increased by more than 50% throughout the occluded bed (p < 0.005) after NG, and was more than the spontaneous increase seen in controls (p < 0.05), but MX had no additional effect on CBF over NG alone. Six-hour infusions of NG therefore decreased infarct size and improved CBF, and addition of MX to reverse the systemic effects of NG did not lessen the benefit. The results suggest that under the conditions of this study, myocardial protection by NG did not depend on a decrease in myocardial oxygen demands, but rather on an increase in collateral flow resulting from a direct vasodilating action of NG on the coronary bed.

INTRAVENTOUS NITROGLYCERIN (NG) has been shown to reduce ST-segment elevation in dogs and man in the early stages of myocardial infarction, and to decrease myocardial necrosis after 5-hour coronary occlusions in dogs. NG dilates systemic and coronary vessels, so the beneficial effects in acute myocardial infarction may be caused by a net reduction of myocardial oxygen demands associated with decreases in afterload and preload, or to increases in collateral flow to ischemic myocardium, or both. As perfusion pressure is a major factor in limiting infarct size and determining collateral flow, correction of NG-induced hypotension and reflex tachycardia with concomitant α-adrenergic agonists has been used and found to result in less ST-segment elevation, and more collateral flow, and less necrosis in dogs. Bache found that NG alone increased flow to transiently ischemic myocardium in conscious dogs, while the combination of NG and phenylephrine-induced hypertension produced even greater increases. These studies emphasize that increasing perfusion pressure may further increase collateral flow and decrease ischemic injury, but they do not establish whether NG alone given after permanent coronary occlusion can significantly increase collateral flow and decrease ultimate infarct size.

We studied the effects of NG on collateral flow and infarct size after permanent coronary occlusion in the conscious dog. NG was given by i.v. infusion for 6 hours after coronary occlusion, and the dose was adjusted so that mean arterial pressure decreased by 10% but stayed above 90 mm Hg. We also studied the effect of restoring the arterial pressure to the level present before NG infusion using a concomitant infusion of methoxamine (MX).

Methods

Fifty-two mongrel dogs that weighed 18–23 kg were instrumented under general anesthesia (sodium pentobarbital, 25–35 mg/kg i.v.) through a left lateral thoracotomy. A mechanical occluder snare was placed around the left circumflex coronary artery just past the first large marginal branch (2–3 cm from the aorta). Plastic catheters were placed in the external jugular vein, internal carotid artery and left atrium, and the distal ends of the catheters and snare were brought out between the shoulder blades through a subcutaneous tunnel. After surgery, penicillin (1 million U) and streptomycin (1 g) were given intramuscularly and catheters were filled with 1000 units of heparin. Studies were performed on the 48 surviving dogs 7–10 days after initial surgery, when all dogs were active and healthy. On the morning of the study, the dogs were allowed to stand in a sling for support and were given morphine (0.3–0.5 mg/kg i.v.) for sedation and analgesia. Lead II of the ECG and left atrial and
aortic pressures (Stratham P23Db) were recorded continuously on a pen recorder.

Myocardial blood flow (MBF) was measured using serial injections of radioactive microspheres, 7–10 μm in diameter, with Tween-80 added and labeled with one of five isotopes: 125I, 141Ce, 85Sr, 86Sr, and 48Sc (3M Company). The microspheres were sonicated mechanically for 5 minutes before each injection, which consisted of 4 × 10^6 microspheres into the left atrium over 10 seconds, followed by a 10-ml saline flush. Reference arterial blood samples were withdrawn at a constant rate of 2.17 ml/min on a calibrated Harvard pump, beginning 30 seconds before microsphere injection and continuing for 2 minutes thereafter. Initial preocclusion MBF was measured about 60 minutes after morphine was given. All dogs were then premedicated with lidocaine, 1 mg/kg i.v., to suppress ventricular ectopic complexes. Three minutes later, the snare was pulled to produce acute permanent occlusion of the LC coronary artery. A second microsphere flow measurement was made 20 seconds after occlusion. The dogs were then randomized into three treatment groups and i.v. infusions were begun 3 minutes after occlusion and continued for 6 hours. The control group consisted of 21 dogs given 150 ml of 0.9% saline. The NG group received NG in 150 ml of 0.9% saline (16 dogs), in a dose sufficient to reduce the mean arterial pressure by 10% below the pre-NG level, but not below 90 mm Hg, followed by an infusion (average 6 μg/kg/min, range 4–10 μg/kg/min) adjusted to maintain the mean arterial pressure at that level.

In another 11 dogs, NG was given as above, but after allowing 3–5 minutes for the mean arterial pressure to level off at 10% below the pre-NG level, MX (20 mg in 1000 ml saline, i.v.) was infused separately (less than 10 ml over 6 hours) to restore the mean arterial pressure to, but not above, the pre-NG level (NG + MX group). In all three groups, further MBF measurements were made 15 minutes, 1 hour, and 6 hours after the infusions were begun. No attempt was made to suppress ectopic activity during the infusion period. Arterial blood samples (10 ml) were taken 3 hours after beginning NG infusions for NG blood levels by gas-liquid chromatography with electron capture detection. After the final flow measurement, the dogs were returned to their cages and brought back 2 days later for ECG and hemodynamic recordings in the conscious state. The 43 dogs surviving 2 days were then given a lethal dose of anesthetic, and the hearts were removed, washed free of blood and weighed.

Postmortem coronary arteriograms were made to delineate the occluded bed or risk region (fig. 1). The coronary arteries (LC, left anterior descending [LAD], and right coronary artery) were cannulated separately at their origins and injected simultaneously under controlled pressure (160 mm Hg) with a barium sulphate-gelatin mass. The volume of the injections was about 5 ml and resulted in a mean weight gain of 3.5 g (range 2–5 g). The viscosity of the injectate was such that there was no penetration beyond the precapillary level. The hearts were packed with gauze to maintain diastolic relationships, fixed in 20% formalin, and radiographed stereoscopically. The hearts were then cut into six transverse sections, the first five being of equal width (1–1.2 cm) from apex to the level of the occlusion near the base, and the sixth including basal myocardium above the occlusion (fig. 2). Pairs of metallic wire markers were placed through the walls at two opposite points in the sections which were then radiographed and photographed with no magnification. Coded arteriograms were viewed stereoscopically by an observer without knowledge of the treatment given or the appearance of the hearts. Completeness of the LC occlusion was confirmed in each heart by finding an abrupt interruption in the arteriogram of the LC vessel, with nonfilling of a short segment. The boundaries of the occluded bed were marked on each radiographed section, using the whole heart radiographs to follow the course of the epicardial vessels.

Each coronary artery branch was identified as originating from a normal vessel or from the circumflex artery distal to the occlusion. The course of each branch was then followed from ring to ring, and a mark was placed where the terminal ramifications of an occluded artery interdigitated with those of an unoccluded vessel. In all hearts the occluded bed filled via collaterals from the nonoccluded vessels. The lateral portion was well opacified, allowing good definition of the occluded bed boundary, although there was a variable zone in the center of the risk region around the posterior papillary muscle that did not opacify fully when infarcts were large. Because of the depth of the sections (1–2 cm), each marked boundary represented an average through the thickness of the ring. The extent of the overlap between occluded and unoccluded beds across these markings was estimated and averaged 1.5 mm; the range was 0.5–1 mm in basal, 1–2 mm in middle, and 1–5 mm in apical sections. Markings of the occluded bed region were made on coded, unmarked copies of the radiographs by a second observer without the stereoscopic viewer and they differed from those of the first observer by −1.1 ± 0.1 mm (se) (430 observations in 43 hearts), indicating good interobserver reproducibility. The difference represented 2% of the width of the risk region at the base and 5% at the apex.

The transverse sections were freed of the right ventricle and fatty and valvular tissue, and the rings of the left ventricle (LV) were weighed. Tracings of each ring and the infarct, identified visually on the unstained, formalin-fixed section, were made on a transparent plastic sheet by the consensus of two observers without knowledge of treatment given. These tracings were then superimposed on the marked radiographs and aligned using the transmural metallic markers as well as natural markers (anterior and posterior papillary muscles, the cavity contour, the septum, and the two junctions with the right ventricle). The arteriographically defined markings of the occluded bed were copied on those tracings and the markings then transferred to the left ventricular rings to facilitate
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Figure 1. (A) A representative example of a postmortem stereoscopic coronary arteriogram of the whole heart. The site of occlusion of the left circumflex (CIRC) coronary artery is indicated by the arrows. The transverse lines show the sections illustrated in B and C. The left anterior descending (LAD), obtuse marginal (MARG), circumflex and right coronary arteries are indicated. (B) Radiograph of a middle transverse section of the heart with radiopaque markers for alignment of tracings. Outlines of the anatomic occluded bed are indicated by dotted lines. The placement of these lines and the regions supplied by the major epicardial coronary artery branches is determined by examining paired radiographs on a stereoscopic viewer. (C) The infarct in the same middle section of the heart.

Sampling for flow. Transmural samples were taken serially from the occluded bed around the posterior papillary muscle so as to include the center of the infarct, the margins of the infarct, within the infarct boundary on the endocardial surface, and the adjoining visually normal border tissue within the occluded bed. Transmural samples were also taken from the center of the nonischemic LAD coronary artery bed near the anterior papillary muscle (fig. 2). The transmural samples were divided into inner and outer halves, which were weighed (range 0.5–1 g in the LC and 1–2 g in the LAD regions), placed in vials containing 10% formalin, and counted for radioactivity together with the reference blood samples in a well-type gamma scintillation counter (Packard #5986) at five energy windows adjusted to the peak emission of the five nuclides. Myocardial blood flow (ml/min/g) was calculated from the formula: MBF = Cm × RBF/Cr, where Cm = corrected counts/g in myocardial samples, RBF = reference blood flow (withdrawal rate of the Harvard pump), and Cr = counts in the reference blood sample. The MBF for each region was found by pooling appropriate myocardial samples. Coronary vascular resistance was calculated by dividing mean flow by mean arterial pressure.

Because of the possibility that any differences found between groups might be related to different degrees of microsphere loss and/or tissue swelling in the infarct zone,22–24 flow values in each region for each time interval were corrected using a factor specific for the individual dog and region. The reduction in content of microspheres injected before occlusion in each ischemic area, expressed as a ratio of radioactive counts per gram in that area to that in nonischemic tissue (I/NI), was used as a quantitative measure of the combined effects of microsphere loss, local edema, hemorrhage, and inflammatory cell infiltrate.24, 25 Underestimation of flow due to these factors was corrected for by dividing each flow by this I/NI ratio (table 1). On the average, slightly greater microsphere loss and/or edema occurred in control compared with NG or NG + MX groups, especially in the outer half of the infarct center and margin regions (table 1).

In previous studies using this canine model, we
assessed the accuracy of flow measurements to be 5-18\% (sd) in the central ischemic region and 3-5\% in nonischemic areas, using pooled samples. These values are based on injections of $4 \times 10^4$ microspheres, resulting in over 1000 spheres in all reference blood samples (usually over 5000) and more than 400 spheres in the inner central ischemic region in about 75\% of experiments. While small sphere numbers are expected to reduce the precision of flow measurements, making it more difficult to show differences between control and treatment groups, they should not create differences where none exist.

Tracings of each left ventricular ring were coded and planimetered electronically by an unbiased technician to determine the areas of LV, infarct, and occluded coronary bed. Areas of the top and bottom surfaces were averaged. The average areas of the infarct and occluded bed were then expressed as ratios of the average area of each ring. The weights of infarct and occluded bed in each left ventricular ring were calculated by multiplying the above ratios by the weights of corresponding LV rings. From these data, the total masses of infarct and occluded bed, or risk region, were calculated in grams for each heart. This planimetric method minimized errors that related to changes in heart weight during processing. The barium injection mass caused a small gain in left ventricular weight, although this was balanced in part by the dehydrating effect of formalin. The slight additional weight was spread over the whole LV and added proportionally to both infarct and occluded bed masses, because both were calculated by summing products of area ratios and left ventricular slice weights. Measurements of the dimensions of the infarct within the occluded coronary bed (fig. 2B) were made in millimeters with fine calipers and were used to reconstruct maps showing the spatial relationship of the infarct within the occluded bed for each LV ring.

FIGURE 2. The technique for sectioning the heart is illustrated. Five sections of the left ventricle were made below the site of the occlusion. From each ring, samples were taken from the center (C) and margins (M) of the infarct (hatched) and its lateral borders (B) around the posterior papillary muscle, and from the center of the nonrisk myocardium around the anterior papillary muscles. The samples were divided into equal epicardial and endocardial halves. Border samples were taken away from necrotic tissue and within the lateral occluded bed boundaries. (B) The method used to spatially reconstruct maps of the infarct within the risk regions, from base to apex of the left ventricle for the three groups. Measurements were made (in mm) within the risk regions along lines numbered 1-13. At lateral borders of the occluded bed, measurements 1 and 11, 3 and 13 were averaged and thicknesses were measured along 4, 5, 9 and 10. Within the infarct, measurements were made at four equal intervals and corresponding values from endocardial (2a to 2d) and epicardial (12a to 12d) surfaces were averaged. The thicknesses of the infarct (6b, 7b, 8b) and the uninfarcted outer rim (6a, 7a, 8a) were measured. LAD = left anterior descending coronary artery; LC = left circumflex artery; LV ring = left ventricular ring.
from base to apex in the three treatment groups.

Formalin-fixed tissue samples from infarcted regions of the middle left ventricular ring of each heart were embedded in paraffin and histologic sections made in the same planes as the planimetered surfaces. The sections were coded, stained with hematoxylin and eosin, and examined independently for the distribution and amount of necrosis by two of us. Estimates of total necrosis per sample, in percent, by the two observers were in close agreement ($y = 0.97x + 5.0, r = 0.95, n = 112$) and differed by an average of $5 \pm 5$% (SD). Percent visual necrosis agreed closely with percent histologic necrosis (visual = 0.95 histologic - 6.7%, $r = 0.93, n = 112$ samples), although visual assessment tended to provide a slight underestimation of total necrosis in the heart.

Paired and unpaired $t$ tests were used to calculate the significance of differences within and between groups. Linear regression analysis was done by the least-squares fit method, and the significance of $r$ values and slopes was calculated. The two-by-two chi-square test was used to assess the significance of differences in event frequency between groups. For the analysis of sequential postocclusion flows in the control group and for the comparison of flows in the control, NG, and NG + MX groups, repeated measures analysis of variance was done for endocardial and epicardial layers with each combination of the infarct center, infarct margin and border regions of the occluded bed.

Results

All 48 conscious dogs survived the first few minutes of LC occlusion, but five developed ventricular fibrillation over the next 5 hours and died. Three of these early deaths were control dogs (three of 18, 17%), one was treated with NG (one of 16, 6%) and one other was treated with NG + MX (one of 11, 9%). These differences in mortality were not statistically significant ($\chi^2 = 0.61$ and 0.18, respectively, for NG and NG + MX groups vs controls, and $\chi^2 = 0.08$ for the NG vs NG + MX groups). Postmortem coronary arteriography was done on all 48 hearts and the size of the occluded coronary bed among the early deaths was within the range of values for the respective groups. In all 48 hearts, the arteriograms were satisfactory, with complete occlusion of the LC coronary artery and filling of the occluded bed via collaterals. Data in the 43 dogs surviving 2 days form the basis for this report: 18 saline-treated, 15 NG-treated and 10 treated with NG + MX.

The hemodynamic changes are summarized in table 2. Resting heart rate, mean arterial pressure, and mean left atrial pressure were similar in the three groups. After coronary occlusion, heart rate and left atrial pressure increased significantly ($p < 0.001$) but mean arterial pressure did not change. These changes were maintained over the next 6 hours in the control dogs. In the NG group, NG produced a 10% reduction in mean arterial pressure (from 115 to 103 mm Hg, $p < 0.001$), sustained over the 6-hour infusion period. Heart rate did not change, except for a transient reflex tachycardia coinciding with the initial drop in mean arterial pressure that lasted for only 15–90 seconds. In the NG + MX group, NG produced similar hemodynamic changes as in the NG group, but MX given promptly to correct the NG-induced hypotension to the pre-NG level abolished the effect of NG on both mean arterial pressure and left atrial pressure. Again, these changes were maintained during the 6 hours of combined NG and MX infusions. The mean arterial pressure remained above 90 mm Hg during the NG infusion; the range over the 6 hours was 90–112 mm Hg in the NG group. In the NG + MX group, mean arterial pressure over the 6 hours ranged from $89 \pm 6$% (mean $\pm$ sd) to $110 \pm 5$% of the pre-NG level.

There was a fairly wide variation in the dose of i.v. NG required to produce the 10% reduction in mean arterial pressure: The total dose ranged from 32–80 mg, the equivalent of 80–200 0.4-mg NG tablets. NG blood levels measured half-way during the 6-hour infusion were high, ranging from 12–33 ng/ml in eight dogs. These levels were greater than those required to reduce mean arterial pressure by 10% in infarction patients (average 1.6 ng/ml; range 0.5–2.7 ng/ml with infusion rate 37.5–175 $\mu$g/min).$^{21}$ Therapeutic levels of NG are less than 20 ng/ml in man.$^{21}$

NG significantly reduced infarct size both as percent LV and as percent of the occluded coronary bed (table 3, fig. 3). Correction of the 10% decrease in mean arterial pressure with MX did not have any additional beneficial effect on infarct size over that of NG alone. Despite occlusions being made at a con-
### Table 2. Hemodynamic Changes in Conscious Dogs Treated with Saline, Nitroglycerin and Nitroglycerin Plus Methoxamine

<table>
<thead>
<tr>
<th>Group</th>
<th>Timing</th>
<th>Heart rate (beats/min)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Mean left atrial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline controls (n = 18)</td>
<td>Preocclusion</td>
<td>104 ± 5</td>
<td>127 ± 3</td>
<td>9 ± 1</td>
</tr>
<tr>
<td></td>
<td>Postocclusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 sec-3 min</td>
<td>127 ± 5†</td>
<td>126 ± 4</td>
<td>13 ± 1§</td>
</tr>
<tr>
<td></td>
<td>5–8 min*</td>
<td>121 ± 4</td>
<td>127 ± 3</td>
<td>13 ± 1</td>
</tr>
<tr>
<td></td>
<td>1 hour</td>
<td>117 ± 2</td>
<td>122 ± 3</td>
<td>10 ± 1</td>
</tr>
<tr>
<td></td>
<td>6 hours</td>
<td>116 ± 3</td>
<td>119 ± 2</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Nitroglycerin (n = 15)</td>
<td>Preocclusion</td>
<td>100 ± 4</td>
<td>121 ± 2</td>
<td>8 ± 1</td>
</tr>
<tr>
<td></td>
<td>Postocclusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 sec-3 min</td>
<td>128 ± 3</td>
<td>115 ± 2</td>
<td>13 ± 2§</td>
</tr>
<tr>
<td></td>
<td>5–8 min*</td>
<td>126 ± 3</td>
<td>103 ± 2*</td>
<td>6 ± 1†</td>
</tr>
<tr>
<td></td>
<td>1 hour</td>
<td>119 ± 4</td>
<td>106 ± 3</td>
<td>7 ± 1</td>
</tr>
<tr>
<td></td>
<td>6 hours</td>
<td>118 ± 5</td>
<td>104 ± 3</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Nitroglycerin plus methoxamine (n = 10)</td>
<td>Preocclusion</td>
<td>106 ± 7</td>
<td>127 ± 2</td>
<td>8 ± 1</td>
</tr>
<tr>
<td></td>
<td>Postocclusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 sec-3 min</td>
<td>124 ± 7†</td>
<td>121 ± 3</td>
<td>12 ± 1§</td>
</tr>
<tr>
<td></td>
<td>5–8 min*</td>
<td>125 ± 6</td>
<td>108 ± 3*</td>
<td>6 ± 1†</td>
</tr>
<tr>
<td></td>
<td>10–15 min†</td>
<td>118 ± 8</td>
<td>123 ± 3**</td>
<td>13 ± 2**</td>
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<td>1 hour</td>
<td>112 ± 7</td>
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<td>13 ± 1</td>
</tr>
<tr>
<td></td>
<td>6 hours</td>
<td>117 ± 10</td>
<td>121 ± 3</td>
<td>15 ± 2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

*Steady state after saline or nitroglycerin alone.
†Steady state after nitroglycerin plus methoxamine.
‡p < 0.001 = significance between preocclusion and postocclusion value.
§p < 0.05, significance between pre- and postnitroglycerin values.
**p < 0.05, significance between postnitroglycerin and postnitroglycerin plus methoxamine values.

### Table 3. Effect of Nitroglycerin and Nitroglycerin with Methoxamine on Infarct Size

<table>
<thead>
<tr>
<th>Infarct mass (g)</th>
<th>Occluded bed mass (g)</th>
<th>LV mass (g)</th>
<th>Infarct/LV (%)</th>
<th>Occluded bed/LV (%)</th>
<th>Infarct/occluded bed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n = 18)</td>
<td>11.5 ± 2.2</td>
<td>32.1 ± 2.3</td>
<td>93.9 ± 3.0</td>
<td>12.1 ± 2.2</td>
<td>34.2 ± 2.2</td>
</tr>
<tr>
<td>NG (n = 15)</td>
<td>6.8 ± 1.7*</td>
<td>36.1 ± 4.0</td>
<td>95.0 ± 5.9</td>
<td>6.4 ± 1.4†</td>
<td>37.1 ± 2.6</td>
</tr>
<tr>
<td>NG + MX (n = 10)</td>
<td>7.0 ± 2.3*</td>
<td>33.3 ± 4.8</td>
<td>100.2 ± 4.5</td>
<td>6.6 ± 2.0†</td>
<td>32.7 ± 3.8</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; range is in parentheses.

* p < 0.2 vs saline group.
† p < 0.05 vs saline group.
‡ p < 0.005 vs saline group.

Abbreviations: LV = left ventricular; NG = nitroglycerin; MX = methoxamine.

stant anatomic site, a wide range of infarct and occluded bed sizes were seen in each group. There was no significant difference in occluded bed mass between saline and NG or NG + MX groups (table 3). Five dogs had no visible infarct (one control, one NG, three NG + MX); occluded bed mass was small in each case, ranging from 17.8–22.9 g, or 17.7–26.4% of the LV.

In all groups the total mass of the infarct was closely related to the mass of the occluded bed or risk region (fig. 4). The slopes of the linear regressions for the NG group and NG + MX group were similar...
Nitroglycerin (NG) and nitroglycerin + methoxamine (NG + MX) reduced infarct size by similar amounts compared with saline controls (C). Infarct size is expressed as percent of the left ventricle and the infarcted bed (risk region). The mass of risk regions and left ventricles did not differ significantly in the three groups \( *p < 0.05; **p < 0.005 \) vs control group). Means ± SEM for the groups are shown.

(0.39 vs 0.46), but the slope was significantly greater in controls (0.90, \( p < 0.001 \)) indicating that infarcts were smaller for any size risk region after NG or NG + MX. Similar results were found when infarcts and occluded beds were normalized for left ventricular mass, i.e., expressed as percent LV. The slopes were 0.98, 0.43, and 0.49 for control, NG, and NG + MX groups, respectively (control vs NG or NG + MX, \( p < 0.001 \); NG vs NG + MX, \( p = \text{NS} \)). The data were also examined to determine whether treatment with NG or NG + MX altered the minimal occluded bed size required for infarction (i.e., the horizontal axis intercept). Inclusion of hearts with small occluded beds and corresponding small infarcts forced the linear regressions for the three groups through a common intercept (fig. 4), so hearts with occluded bed mass \(<30 \text{ g}\) or \(30\%\) of the LV were arbitrarily excluded for this analysis. Expressed in terms of absolute mass, the horizontal intercept was smaller in controls than in NG or NG + MX groups, but the differences were not statistically significant. Expressed as percent of LV, the intercepts were 23.4%, 34.3%, and 32.0% for control, NG and NG + MX groups, respectively (control vs NG, \( p < 0.005 \); control vs NG + MX, \( p < 0.05 \); NG vs NG + MX, \( p = \text{NS} \)). After exclusion of hearts with small occluded beds, the differences in slope between control and treatment groups were no longer statistically significant.

NG and NG + MX produced similar alterations in the spatial relationship between infarcts and corresponding occluded beds in individual LV rings (fig. 5). In all three groups, both the infarct and risk region were largest in basal and middle portions of the heart and tapered toward the apex. The infarct/risk region ratio was greater in the basal than in the apical rings in the three groups. NG decreased the absolute mass of the infarct per ring \( (p < 0.05 \) vs controls) as well as the infarct/risk region ratio. The reconstructed maps

**Figure 3.** Nitroglycerin (NG) and nitroglycerin + methoxamine (NG + MX) reduced infarct size by similar amounts compared with saline controls (C). Infarct size is expressed as percent of the left ventricle and the infarcted bed (risk region). The mass of risk regions and left ventricles did not differ significantly in the three groups \( *p < 0.05; **p < 0.005 \) vs control group). Means ± SEM for the groups are shown.

**Figure 4.** Infarct size was directly related to the size of the occluded bed (risk region). (left) Nitroglycerin (NG) significantly altered the slope of the relation in controls (C) so that there was less infarct for a risk region greater than about 20 g. (right) NG plus methoxamine (NG + MX) had an effect similar to NG alone.
(fig. 5) in dogs with infarcts from the three groups show that there is a significant rim of uninfarcted myocardium within the occluded bed both laterally and subepicardially. On comparing the maps for the three groups, it is apparent that NG and NG + MX reduced infarct size in both lateral and subepicardial directions throughout the occluded bed.

Regional MBF data were available in 32 of the 43 dogs: 13 in the saline group, 12 in the NG group and seven in the NG + MX group. Flows from six dogs of the original 43 were excluded because of arrhythmias during flow measurements. The other five dogs had small occluded beds and no infarcts and were analyzed separately.

In all dogs, MBF decreased throughout the occluded bed after coronary occlusion. In the 33 dogs with infarcts, this decrease averaged 86% in the center of the infarct or occluded bed (0.98 ± 0.05 to 0.14 ± 0.01 ml/min/g), 71% in the margin of the infarct (1.00 ± 0.05 to 0.29 ± 0.03 ml/min/g), and 29% in the normal appearing border area within the occluded bed (0.99 ± 0.05 to 0.70 ± 0.05 ml/min/g). In the five dogs with small risk regions, there was only a 42% decrease in flow to the center of the occluded bed (1.18 ± 0.21 to 0.69 ± 0.14 ml/min/g) and the postocclusion flows were higher than in the 33 dogs with infarcts (0.69 vs 0.10 ml/min/g, p < 0.001). There was a gradient of collateral flow from lateral to central regions of the occluded bed, and epicardial flows were greater than endocardial flows throughout the risk region. The inner/outer ratios in the 33 dogs preocclusion and at 20 seconds postocclusion were 1.04 ± 0.03 vs 0.38 ± 0.04 in the infarct center, 1.02 ± 0.03 vs 0.52 ± 0.04 in the infarct margin, 1.05 ± 0.03 vs 0.74 ± 0.04 ml/min/g in the normal border and 1.04 ± 0.03 vs 1.03 ± 0.03 ml/min/g in the nonischemic LAD region.

The comparability of 20-second postocclusion flows in control, NG, and NG + MX groups was tested for each region within the occluded bed using the multiple comparisons least significant difference (LSD) test based on the analysis of variance (fig. 6). Of the 12 possible comparisons, none of the differences were significant except for three that were significant at the p < 0.05 level: in the outer infarct margin, NG + MX exceeded control, while in the border region (inner and outer) control was greater than NG. Because multiple comparisons were performed, we feel there were probably no physiologically important differences between the three groups at 20 seconds postocclusion in the infarct center and margin regions. In the border area higher flows in control dogs may be explained by the fact that border samples were taken lateral to the infarct; infarcts were larger in control animals, so the tissue sampled was closer to the lateral boundary of the occluded bed.

In the control dogs with visible infarcts, collateral blood flow increased throughout the occluded bed in the first 15 minutes postocclusion, but did not change significantly between 15 minutes and 6 hours. Analysis of variance showed the increase from 20 seconds to 6 hours to be significant in all regions in the control group (fig. 6). In the NG group, collateral flow also increased significantly in all regions. However, the in-
increase over the first 15 minutes was greater than control, and unlike the control group, flow continued to increase over the 6-hour infusion period (fig. 6). In the NG + MX group, the pattern of flow increase was similar to NG alone in the infarct center and margin, but quantitatively less; because of the small number of dogs and large variance, the increases were not statistically significant. In the border region, flow changes in the NG + MX group over time were minimal.

The three groups were compared at 6 hours using the multiple comparisons LSD test (fig. 6). NG was significantly higher than control ($p < 0.05$) in all regions, while NG + MX exceeded control only in the infarct center and margin regions. Comparing NG and NG + MX gave mixed results. NG was significantly higher than NG + MX in the outer half of the infarct center, inner half of the infarct margin and border region, but not in the inner half of the infarct center or outer half of the infarct margin (fig. 6).

Flow in the nonoccluded LAD bed did not change significantly over the 6 hours in controls (table 4). However, in the NG group, LAD flow decreased between 20 seconds and 15 minutes ($p < 0.05$),

### Table 4. Flow in Nonischemic Area in the Three Groups of Dogs with Infarction

<table>
<thead>
<tr>
<th>Timing</th>
<th>Saline controls (n = 13)</th>
<th>Nitroglycerin (n = 12)</th>
<th>Nitroglycerin + methoxamine (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inner</td>
<td>Outer</td>
<td>Inner</td>
</tr>
<tr>
<td>Preocclusion</td>
<td>1.03 ± 0.05</td>
<td>1.06 ± 0.08</td>
<td>0.86 ± 0.07</td>
</tr>
<tr>
<td>Postocclusion</td>
<td>1.01 ± 0.06</td>
<td>1.08 ± 0.07</td>
<td>0.99 ± 0.07</td>
</tr>
<tr>
<td>20 sec</td>
<td>1.26 ± 0.10</td>
<td>1.20 ± 0.09</td>
<td>0.85 ± 0.07</td>
</tr>
<tr>
<td>1 hour</td>
<td>1.02 ± 0.06</td>
<td>1.03 ± 0.05</td>
<td>1.24 ± 0.14</td>
</tr>
<tr>
<td>6 hours</td>
<td>1.04 ± 0.09</td>
<td>1.06 ± 0.09</td>
<td>1.23 ± 0.13*</td>
</tr>
</tbody>
</table>

Flow is measured as ml/min per gram.

*Changes in flow over time significant in NG group (see text).
probably because of the reduced myocardial oxygen demands associated with the 10% fall in arterial pressure after NG; it is important to note that collateral flow in the occluded bed was increasing at the same time. The LAD flows at 1 hour and 6 hours were higher than at 15 minutes. In the NG + MX group, LAD flows did not change significantly and were similar to those in the control group.

The calculated changes in regional coronary vascular resistance paralleled those in regional MBF in all three groups. The resistances were reduced significantly in NG and NG + MX groups throughout the occluded bed with gradients from lateral to central regions and epicardial to endocardial regions.

In the five dogs with small occluded beds and no infarcts, collateral blood flows in the center of the occluded bed at 20 seconds, 15 minutes, 1 hour and 6 hours after occlusion were 0.69 ± 0.14, 0.78 ± 0.14, 1.02 ± 0.21 and 0.76 ± 0.06 ml/min/g, respectively. Because there was only one dog each in the control and NG groups and three dogs in the NG + MX group, separate analysis of the amounts of increase in flow in the three groups was not meaningful.

The relation of collateral flow to myocardial salvage is shown in figure 7. Collateral flow 6 hours postocclusion, expressed as a ratio of corresponding LAD flow, was directly related to the amount of salvage of myocardium in the occluded bed for all dogs (y = 0.02x - 0.87, r = 0.85, n = 38, p < 0.001). Inspection of the figure suggests that the relation may be a continuous, curvilinear one, although the regression coefficient for an exponential fit was not superior to the linear coefficient (0.87 vs 0.85). A similar relationship was seen between myocardial salvage and collateral flow at 1 hour.

Discussion

There were two major findings in this study. First, NG reduced infarct size when arterial pressure was kept above 90 mm Hg, the fall in arterial pressure was mild (≤ 10%), and there was no sustained reflex tachycardia. In this setting, the addition of MX to abolish the small decrease in arterial pressure did not modify the effect of NG on infarct size. These findings are consistent with those of Myers et al.3 and Epstein et al.,4 who reported that NG and NG + MX reduced ST-segment elevation in models of acute myocardial infarction, even with simulation of multivessel disease, and that myocardial ischemia was less when heart rate after NG alone increased by less than 50% of control but was more when heart rate increased by more than 50% of control. Our findings do not exclude the possibility that the addition of MX might have been more beneficial than NG alone if there had been reflex tachycardia and greater hypotension after NG.

Second, NG produced a marked increase in collateral flow over the 6-hour infusion period. Addition of MX appeared to result in some attenuation of this increase in certain areas, particularly in higher flow regions within the occluded bed. Previous investigators have found that NG can increase collateral flow during acute myocardial ischemia,10, 12, 14, 17-19 and that the addition of MX can augment this response.16, 18 The failure of MX to provide an additive effect in our study may have been due to the relatively small decrease in aortic pressure with NG alone, the MX-induced increase in LV filling pressure resulting in possible subendocardial vascular compression, or a direct α-adrenergic-mediated constriction of coronary vessels. The latter mechanism is supported.

![Figure 7. Collateral flow in the center of the risk region 6 hours after occlusion was directly related to the amount of myocardial salvage within the risk region in dogs with infarction. The linear regression curve for all three groups is shown (y = 0.02x - 0.87, r = 0.85, n = 38, see = 0.18, p < 0.001).](http://circ.ahajournals.org/doi/abs/10.1161/01.CIR.63.1.26?journalCode=cir)
by the finding that MX attenuated the response to NG in higher flow areas rather than low-flow areas within the occluded coronary bed. Alpha-adrenergic receptors have been identified in both large and small coronary arteries,18, 29-30 although not in intercoronary collaterals,18 and methoxamine has recently been shown to constrict epicardial coronary arteries in the conscious dog.31

The explanation for the early increase in collateral flow during NG infusion is uncertain. Vessels within the occluded bed are believed to become maximally dilated soon after coronary occlusion and should have been unresponsive to NG, although other sites of action were possible, including the collateral vessels. Diminished LV filling and subendocardial compressive forces resulting from systemic effects of NG may have been important; however, increased flow still occurred when systemic effects of NG were reversed by MX. The progressive increase in flow that occurred within NG between 15 minutes and 6 hours also requires explanation. One possibility is that NG accumulated in the blood or that there was a buildup of some other vasoactive compound. Release of immunosassayable prostaglandin E by NG has been demonstrated,32 and PGE, is a well-known coronary and systemic vasodilator. Another possibility is that the early NG-induced increase in flow retarded necrosis sufficiently to allow subsequent flow increases to occur by “natural” processes.

Unfortunately, flow was not measured after NG was stopped, so it is uncertain whether the increase was sustained beyond the 6-hour infusion period. However, in light of the associated long-term myocardial salvage, it is likely that the increase in fact persisted. Capurro et al.32 found that the combination of NG + MX begun 10 minutes after coronary occlusion and continued for 1 hour in closed-chest, sedated dogs resulted in a sustained increase in collateral flow that lasted for at least 5 hours. Once collaterals have been opened up by NG, they may remain open after NG is discontinued.

By comparing the results in our NG and NG + MX groups we must conclude that the major factor producing myocardial salvage in our study was the increase in collateral blood flow. Reversal of NG-induced hypotension by MX, with concomitant increase in myocardial oxygen demands, did not eliminate the beneficial effect of NG on salvage. The failure of MX to increase infarct size compared with NG alone may be explained by the relative unimportance of the small changes in myocardial oxygen demands we produced. In man, myocardial oxygen demands may be much more important. In patients with acute myocardial infarction, phenylephrine infusion has been shown to completely reverse the improvement in hemodynamics and precordial ST-segment elevation resulting from i.v. NG.37

In a 24-hour canine infarction model, Fukuyama and Roberts39 did not find a significant reduction in infarct size with NG infusions over 8 hours after coronary occlusion. Possible explanations for their different findings may be: (1) greater decreases in mean arterial pressure after NG (15%) and to lower levels (80 mm Hg in some dogs); (2) higher heart rates; (3) much smaller increases in collateral blood flow (0.06–0.09 ml/min/g); and (4) the different method of analysis of infarction, in which the size of the occluded coronary bed was not taken into account.

Questions have been raised about the validity of microsphere measurements of flow in regions of infarcted myocardium due to apparent loss of spheres.22, 23 Recently, this apparent loss was found to result from both true physical dropout of spheres with trapping in lung and regional lymph nodes as well as weight gain by infarct tissue from edema, inflammation and/or hemorrhage with “dilution” of microsphere counts.23, 24 As advocated by Cobb et al.,25 we used the severity of depression of preocclusion microsphere content in each region to correct for the combined effects of weight gain and true sphere loss. This analysis assumes that within each region the apparent loss of each set of spheres injected pre- and postocclusion is similar. The microspheres were injected over a limited period of time (6 hours), and the dogs were not sacrificed until 42 hours later, so the assumption appears reasonable.

Our model used permanent occlusions of the mid-circumflex coronary artery to achieve a sufficiently large infarct (average 12.1 ± 2.2% LV, range 0–41%) without an unacceptably high mortality. With more proximal circumflex occlusions in conscious dogs, somewhat bigger infarcts were found by Rivas et al.34 (average 19 ± 3% LV, range 4–33%) and Bishop et al.26 (average 15 ± 2% LV, range 0–35%). The smaller infarcts in our study were generally associated with higher levels of collateral flow. One could argue that with more distal occlusions and higher flows, myocardial salvage with NG might have been more readily achieved than in other models with larger ischemic areas. On the other hand, because our control infarcts were relatively small, salvage with NG should have been more difficult to demonstrate. Figures 4 and 5 suggest that NG produced myocardial salvage over a wide range of ischemic zone sizes.

As we have found in previous studies using this model,36, 36, 37 a substantial rim of normal looking myocardium was identified around the infarct within the boundaries of the occluded bed (fig. 5). Forty-eight samples, representing about 20% of all of the samples taken from this area, were examined histologically. Forty-one had no necrosis, while two had 2% necrosis, three had 5% necrosis and two had 10% necrosis (mainly contraction band in type). Microsphere flow measurements indicated moderately, but not severely, reduced perfusion in this area after coronary occlusion. We believe this area represents part of a substantial border zone located both laterally and epicardially within the occluded bed; tissue located in this zone is jeopardized but has potential for long-term salvage. In support of this concept, treatment with NG or NG + MX resulted in infarcts that were smaller in all directions; i.e., they were less transmural and occupied less of the left ventricular circumference, even at the endocardial surface. Similarly, in a previous study
with indomethacin, infacts became larger in all directions.  

In summary, NG increased collateral blood flow and decreased subsequent infarct size after permanent coronary occlusion in conscious dogs in the setting of mild hypotension and no sustained reflex tachycardia. Coronary blood flow appears to have been the major factor in the myocardial salvage effect of NG in this setting. Further clinical studies are needed to determine whether NG can reduce infarct size and, more important, reduce mortality and morbidity from acute infarction in man.

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Effect of intravenous nitroglycerin on collateral blood flow and infarct size in the conscious dog.
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