Effect of Nitroglycerin and Nifedipine on Subendocardial Perfusion in the Presence of a Flow-Limiting Coronary Stenosis in the Awake Dog

Robert J. Bache and Bruce A. Tockman
From the Department of Medicine, Cardiovascular Section, University of Minnesota Medical School, Minneapolis, Minnesota

SUMMARY. This study examined the effects of nitroglycerin and nifedipine on the transmural distribution of myocardial blood flow when ischemia-induced vasodilation of the distal coronary vasculature caused a proximal coronary stenosis to become flow-limiting. Studies were performed in chronically instrumented awake dogs with electromagnetic flowmeter probes and hydraulic occluders on the left circumflex coronary artery. Myocardial blood flow was estimated with 15µ radioactive microspheres. During control conditions, subendocardial (endo) flow significantly exceeded subepicardial (epi) flow (endo:epi = 1.33 ± 0.12). Following a 10-second coronary occlusion, reactive hyperemia occurred with excess arterial inflow resulting in 330 ± 37% blood flow debt repayment. This response was not altered by nitroglycerin (0.015 mg/kg, iv), but was decreased by nifedipine (0.01 mg/kg, iv) to 175 ± 38%, indicating depression of the coronary vasodilator response to a brief ischemic stimulus. When, following a 10-second occlusion, arterial inflow was limited to the preocclusion rate by a proximal stenosis, subepicardial flow increased at the expense of hypoperfusion of the subendocardium (endo/epi decreased to 0.50 ± 0.04; \( P < 0.01 \)), and the continuing subendocardial ischemia resulted in augmentation of the subsequent reactive hyperemia (debt repayment = 556 ± 5%; \( P < 0.01 \)). Both nitroglycerin and nifedipine abolished the augmentation of the reactive hyperemic response which occurred when a total occlusion was followed by an interval of coronary stenosis. This effect was associated with enhanced subendocardial blood flow during the interval of restricted inflow, suggesting that both of these agents alleviate the subendocardial hypoperfusion and ischemia which occur in the presence of a proximal flow-limiting coronary stenosis. (Circ Res 50: 678–687, 1982)

IN the presence of occlusive coronary artery disease, angina pectoris may occur when myocardial metabolic demands transiently exceed the ability of the diseased coronary vessels to deliver arterial inflow. The consequent myocardial ischemia causes vasodilation of the coronary resistance vessels; the resultant loss of vasomotor activity at the microvascular level and the subsequent decrease in coronary perfusion pressure distal to the stenotic region compromises the ability to perfuse the subendocardium (Ball and Bache, 1976; Bache et al., 1977; Rouleau et al., 1979). Antianginal agents with vasodilator properties such as nitroglycerin or the calcium channel blockers may exert a therapeutic effect by reducing left ventricular systolic tension and thereby decreasing myocardial oxygen requirements (Robinson, 1968; Atterhog et al. 1975). The ability of these agents to enhance blood flow to potentially ischemic myocardium is less clear. Increased delivery of blood to ischemic myocardium could be effected either by increasing collateral inflow to the ischemic area or by causing a transmural redistribution of perfusion to favor blood flow to the subendocardium where vulnerability to ischemia is greatest.

We have previously found that if distal coronary vasodilation is produced by a brief period of myocardial ischemia and subsequent coronary flow is limited by a proximal stenosis, subendocardial underperfusion may be perpetuated despite a normal net volume of arterial inflow (Bache et al., 1974). The present study was designed to determine whether nitroglycerin or nifedipine may act to correct this subendocardial underperfusion which occurs during ischemia-induced vasodilation in the presence of a proximal flow-limiting coronary artery stenosis. The transmural distribution of myocardial perfusion was examined with radionuclide-labeled microspheres, while the degree of myocardial ischemia was assessed from the magnitude of the reactive hyperemia following brief periods of coronary occlusion. In addition to these studies of nitroglycerin and nifedipine on subendocardial perfusion in the presence of a flow-limiting coronary stenosis, the effect of these agents on coronary collateral flow into ischemic myocardial areas distal to a total coronary occlusion was examined. Studies were carried out in chronically instrumented awake dogs to eliminate possible interfering effects associated with general anesthesia or acute surgical trauma.

Methods

Twenty-one adult mongrel dogs weighing 18.5–30.0 kg were anesthetized with sodium pentobarbital (25 mg/kg, iv) and underwent left thoracotomy in the 4th intercostal space.
A heparin-filled polyvinyl chloride catheter, 3.0 mm o.d., was introduced into the ascending aorta via the left internal thoracic artery. The heart was suspended in a pericardial cradle, and similar catheters were introduced into the left atrium via the atrial appendage and the left ventricle via a stab wound in the apical diaphragm. The catheters were secured in place with purse string sutures. The proximal 1.5 cm of the left circumflex coronary artery was dissected free and an electromagnetic flowmeter probe (Gould-Statham Instruments, Inc.) was positioned proximal to any branches. A pneumatic occluder, constructed of polyvinyl chloride tubing (2.7 mm o.d.), was placed around the coronary artery just distal to the flowmeter probe and proximal to any arterial branches (Debely, 1971). The catheters, hydraulic occluder tube, and electromagnetic flowmeter leads were tunneled dorsally into a subcutaneous pouch at the base of the neck. On the morning prior to study, the catheters, occluder tube, and flowmeter leads were exteriorized through a 1-cm skin incision using 2% lidocaine infiltration anesthesia. Coronary flow was measured with a Gould-Statham 5P2302 electromagnetic flowmeter. Aortic, left ventricular, and left atrial pressures were measured with Gould-Statham P23Db pressure transducers. Flowmeter calibrations were performed by passing measured flows of saline through the probes. Lead II of a standard electrocardiogram was obtained. Data were recorded on a Hewlett-Packard model 8800 eight-channel direct-writing oscillograph.

Studies were carried out 8–15 days after the initial surgery. At the time of study, the animals were active and fully recovered from the effects of surgery without fever, anemia, or evidence of ill health. The dogs were trained to lie quietly on their right sides during the study. The laboratory was dimly illuminated and kept free of noise or other activity which might disturb the dog. After all recording instruments were connected, a 60-second coronary artery occlusion was produced while lead II of the electrocardiogram was monitored. Only dogs that exhibited greater than 0.1 mV 5-T segment elevation during occlusion, as well as increased heart rate and increased left atrial pressure, were used for this study. Four of the dogs tested failed to manifest these indications of myocardial ischemia during a 60-second coronary occlusion and were, consequently, not included in this study. Subsequently, a 45- to 60-minute interval was allowed for the animal to adjust to the laboratory conditions. During this time, hemodynamic data were sampled continuously to ensure that a control steady state had been achieved.

Group I

Group I consisted of seven dogs with electromagnetic flowmeter probes and hydraulic occluders on the left circumflex coronary artery. During resting control conditions, the reactive hyperemic response after a 10-second coronary artery occlusion was observed. The reactive hyperemic response then was observed when a 10-second total occlusion was performed and a sufficient volume of water was subsequently withdrawn from the occluder to allow coronary flow to return to the pre-occlusion rate and held at this level for either 20 or 60 seconds. After this interval of restricted inflow, the occluder was completely released to allow the reactive hyperemic response. Studies were discarded if coronary flow during the interval of restricted inflow deviated from the pre-occlusion control rate by more than 5%. The sequence in which occlusions were performed was randomized, and each intervention was performed in duplicate.

Measurements of regional myocardial blood flow were made by injecting into the left atrium carbonized microspheres 15 μm in diameter labeled with γ-emitting radio-nuclides 125I, 111In, 85Sr, 51Cr, 95Nb, or 46Sc (3M Company). Microspheres were obtained as 1.0 mCi of each nuclide in 10 ml of 10% dextran. Before injection, the microspheres were mixed for at least 15 minutes in an ultrasonic bath and a vortex agitator. During each intervention, 3 \times 10^6 microspheres were injected into the left atrium over a 5-second interval and flushed in with 3.0 ml of normal saline. Beginning 5 seconds prior to the microsphere injection and continuing for 90 seconds, a reference sample of arterial blood was collected from the aortic catheter at a constant rate of 15.0 ml/min with a withdrawal pump. In each animal, 3 \times 10^6 microspheres were administered during resting control conditions. To evaluate the distribution of myocardial blood flow when coronary vasodilation was produced by a 10-second total coronary occlusion but subsequent coronary inflow was prevented from increasing above the control level, a 10-second coronary occlusion was performed and the occluder then was partially deflated to allow coronary flow to return to the pre-occlusion control rate but to prevent any reactive hyperemia. Beginning 10 seconds after restoration of coronary artery inflow to the control level, microspheres labeled with a second radionuclide were injected into the left atrium. Coronary blood flow was maintained at the pre-occlusion rate for an additional 50 seconds to ensure complete dispersion of microspheres before the occluder was completely released.

After these control measurements had been completed, nitroglycerin (0.015 mg/kg) dissolved in 3.0 ml of normal saline was infused intravenously over a 60-second interval. This dosage was chosen to produce an approximately 2½-fold increase in coronary blood flow. Five minutes after the nitroglycerin infusion was begun, the reactive hyperemic response to a 10-second coronary occlusion was observed. Three minutes later, the reactive hyperemic response to a 10-second coronary occlusion followed by a 20-second restriction of coronary inflow to pre-occlusion rate was again observed. The sequence in which these two interventions were performed was randomized. After a 30- to 45-minute interval, nitroglycerin, 0.015 mg/kg, was again administered over a 60-second interval by intravenous infusion. Five minutes after administration of nitroglycerin, the reactive hyperemic response to a 10-second coronary occlusion followed by a 60-second restriction of coronary inflow to pre-occlusion control rate of flow was observed. To evaluate the effect of nitroglycerin on the transmural distribution of perfusion in the presence of a flow-limiting stenosis, microspheres were injected 10 seconds after restoration of coronary artery inflow to the control rate.

On the following day, the dog was returned to the laboratory for study of the effects of nifedipine on myocardial blood during ischemia-induced coronary vasodilation. Control measurements were obtained of the reactive hyperemia following a 10-second coronary artery occlusion and 10-second coronary occlusions followed by 20- or 60-second periods of coronary stenosis during which coronary inflow was restricted to the control pre-occlusion rate of flow. Myocardial blood flow measurements were then performed with microspheres during control conditions and during ischemia-induced coronary vasodilation 10 seconds after partial release of a 10-second coronary artery occlusion to the pre-occlusion control rate of flow. Subsequently, nifedipine, 0.010 mg/kg, was administered intravenously over a 60-second interval. This dosage of nifedipine was chosen, since it provided a similar peak increase in coronary...
blood flow to that produced by nitroglycerin. Thirty minutes after administration of nifedipine, when coronary blood flow measured with the electromagnetic flowmeter had returned to the pre-occlusion control level, reactive hyperemic responses were observed following 10-second total coronary occlusions, as well as 10-second coronary occlusions followed by 20- and 60-second intervals of coronary stenosis during which arterial inflow was limited to the pre-occlusion control rate. The sequence of coronary occlusions was randomized, and each coronary occlusion was performed in duplicate. To evaluate the distribution of myocardial blood flow when coronary vasodilation was produced but coronary inflow was prevented from increasing above the control level, a 10-second total coronary occlusion was produced and the occluder was partially deflated to allow coronary inflow to return to the preocclusion rate but to prevent reactive hyperemia. Ten seconds after restoration of coronary inflow to the control rate, microspheres were again injected and coronary flow was maintained at the pre-occlusion rate for an additional 50 seconds.

**Group II**

Group II consisted of six dogs with electromagnetic flowmeter probes and hydraulic occluders on the proximal left circumflex coronary artery, as well as catheters in the ascending aorta and the coronary sinus. These dogs were used to study the effects of nifedipine on myocardial oxygen consumption. Duplicate specimens of aortic and coronary sinus blood were drawn anerobically for measurement of $P_{O_2}$, $P_{CO_2}$, and pH with an Instrumentations Laboratory model 113 Blood Gas Analyzer at 37°C. Arterial and coronary sinus oxygen content were calculated from the $P_{O_2}$ and pH determinations, using the $O_2$ dissociation curve for dog blood to estimate $O_2$ saturation, as described by Case and Greenberg (1976). Myocardial oxygen consumption was computed as the product of flow measured in the left circumflex coronary artery and the difference in oxygen content between aortic and coronary sinus blood. After duplicate control measurements were obtained in the resting awake state, nifedipine (0.010 mg/kg, iv) was administered. Thirty minutes after administration of nifedipine, duplicate blood specimens were again obtained from the aortic and coronary sinus catheters for determination of myocardial oxygen consumption. Hemodynamic measurements were recorded continuously during the course of the study.

**Group III**

Group III consisted of eight dogs with electromagnetic flowmeter probes and hydraulic occluders on the proximal left circumflex coronary artery. These animals were used to study the effects of nitroglycerin and nifedipine on regional myocardial blood flow during total coronary artery occlusion. While the animals were resting quietly, 3 X $10^6$ microspheres were injected into the left atrium so that we might examine myocardial blood flow during control conditions. After this injection, the left circumflex coronary artery occluder was inflated while the signal from the electromagnetic flowmeter was monitored to ensure total occlusion. Sixty seconds after application of total coronary artery occlusion, microspheres labeled with a different radionuclide were injected for examination of coronary collateral blood flow. The coronary occlusion was maintained for a total duration of 2 minutes to ensure complete dispersion of microspheres before the occlusion was released. Ten to 40 minutes after this intervention, nitroglycerin (0.015 mg/kg) was administered intravenously over a 60-second interval. Five minutes after beginning the nitroglycerin infusion, the coronary occlusion was repeated and microspheres were again injected 60 seconds after the onset of the occlusion. The occlusion was maintained for a total duration of 2 min.

On the following day, the dogs were returned to the laboratory for study of the effects of nifedipine on coronary collateral blood flow. While the animals were resting quietly, an initial injection of microspheres was again performed to examine control myocardial blood flow. Subsequently, microspheres were injected during a 2-min coronary occlusion to examine control coronary collateral blood flow. Nifedipine (0.010 mg/kg) was then administered intravenously over a 60-second interval. Thirty minutes after administration of nifedipine, a 2-minute coronary occlusion was again performed with injection of microspheres 60 seconds after the onset of occlusion. After completion of the study, the animal was killed with a lethal dose of pentobarbital and the heart removed, weighed, and fixed in 10% buffered formalin. The atria, great vessels, right ventricle, and large epicardial and endocardial blood vessels were dissected from the left ventricle and discarded. Duplicate full thickness blocks were removed from the posterior left ventricular wall, including the posterior papillary muscle region, while duplicate myocardial specimens from the anterior left ventricular wall served as controls. Since injections of methylene blue into the circumflex coronary artery demonstrated myocardial staining within a distribution encompassing the posterior tissue blocks, these specimens were assumed to represent the myocardial regions under study. Each tissue block was divided into four transmural layers of equal thickness from epicardium to endocardium, weighed, and placed in the vials for counting. The transmural layers were designated layers 1 through 4, with layer 1 the most epicardial and layer 4 the most endocardial layer.

Myocardial and blood reference specimens were counted in a Packard model 5912 gamma-counter with multi-channel analyzer at window settings corresponding to the primary emission peaks of each radionuclide. The counts/min recorded in each energy window were corrected for background activity and for overlapping counts from the accompanying isotopes with a digital computer (Domenech et al., 1969). Blood flow (ml/min) to each myocardial specimen was computed using the formula: $Q_m = Q_r \times C_m/C_r$, where $Q_m = $ myocardial blood flow (ml/min), $Q_r = $ reference blood flow (ml/min), $C_m = $ counts/min of the myocardial specimen, and $C_r = $ counts/min of the reference blood sample. Blood flow to each myocardial specimen was then divided by sample weight and expressed as ml/min per g of myocardium.

Measurements of individual reactive hyperemic responses were discarded if heart rate or aortic blood pressure differed by more than 5% during the occlusion and to the end of the reactive hyperemic response. The volume of blood flow during reactive hyperemia was determined by electrical integration of the electromagnetic flowmeter tracing. The duration of reactive hyperemia was taken as the time required for flow to fall within 5% of the control measurement. Heart rate and aortic, left atrial, and left ventricular pressures were measured directly from the recordings. Calculations of blood flow debt, reactive hyperemia flow and blood flow debt repayments were made as described by Olsson and Gregg (1965b). Blood flow debt (ml) = control flow rate (ml/sec) X duration of occlusion (sec), reactive hyperemia flow (ml) = total flow during reactive hyperemia (ml) — [control flow rate (ml/sec) X 60].
Hemodynamic Variables during Control Conditions, following Administration of Nitroglycerin (0.015 mg/kg, iv), and following Administration of Nifedipine (0.01 mg/kg, iv) for Seven Dogs in Group I

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Nitroglycerin</th>
<th>Nifedipine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10-Second occlusion</td>
<td>Post-occlusion restriction</td>
<td>10-Second occlusion</td>
</tr>
<tr>
<td>Heart rate</td>
<td>81 ± 7</td>
<td>82 ± 7</td>
<td>87 ± 12</td>
</tr>
<tr>
<td></td>
<td>(beats/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean aortic pressure</td>
<td>83 ± 5</td>
<td>83 ± 6</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV systolic pressure</td>
<td>99 ± 3</td>
<td>102 ± 4</td>
<td>102 ± 4</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left atrial pressure</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Measurements are shown prior to production of a 10-second coronary occlusion, and prior to post-occlusion restrictions when a 10-second occlusion was followed by a 20- or 60-second interval during which flow was limited to the pre-occlusion level.

There was no statistically significant difference between any values during control conditions and following nitroglycerin or nifedipine.

Results

Group I

Mean heart rate, aortic and left atrial pressures, and blood flow through the left circumflex coronary artery for seven dogs in group I are shown in Table 1. Within 60 seconds after administration of nitroglycerin, coronary blood flow increased from a control of 40.4 ± 4.1 to 117 ± 16.2 ml/min (P < 0.01); this increase was transient so that coronary flow had returned to the control value within 3 minutes after administration of nitroglycerin. Five minutes after nitroglycerin, when microsphere measurements of regional perfusion and examination of the reactive hyperemic response were carried out, heart rate, aortic and left atrial pressures, and coronary flow had returned to the control level. These variables did not change significantly prior to production of 10-second coronary occlusions or post-occlusion restrictions (Table 1). Administration of nifedipine resulted in a more prolonged increase in coronary artery blood flow, which was also accompanied by a significant decrease in aortic pressure and increase in heart rate (Table 2). At the time that microsphere measurements of regional myocardial perfusion and examination of the reactive hyperemic responses were carried out (30

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate, Mean Aortic Pressure, Mean Left Atrial Pressure, and Left Circumflex Coronary Artery Blood Flow in Seven Dogs in Group I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart rate (beats/min)</td>
</tr>
<tr>
<td>Control</td>
<td>82 ± 6</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>129 ± 13*</td>
</tr>
<tr>
<td>5 min</td>
<td>85 ± 9</td>
</tr>
<tr>
<td>Control</td>
<td>82 ± 6</td>
</tr>
<tr>
<td>Nifedipine</td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>129 ± 16*</td>
</tr>
<tr>
<td>5 min</td>
<td>95 ± 10*</td>
</tr>
<tr>
<td>10 min</td>
<td>79 ± 7</td>
</tr>
<tr>
<td>30 min</td>
<td>76 ± 7</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Measurements were obtained during control conditions, during the peak response, and 5 minutes after administration of nitroglycerin, 0.015 mg/kg, iv, as well as following administration of nifedipine 0.010 mg/kg, iv.

* Indicates P < 0.05 in comparison with the respective control measurement.
minutes after nifedipine), heart rate, aortic and left atrial pressures, and coronary flow had returned to the control level. These variables did not change significantly prior to production of 10-second coronary occlusions or post-occlusion restrictions (Table 1).

Figure 1 illustrates the coronary reactive hyperemic response observed in a typical dog, while Table 3 summarizes the reactive hyperemia data from all seven dogs in group I. During control conditions, a 10-second coronary occlusion was followed by a reactive hyperemic response lasting 27.2 ± 4.1 seconds, during which time, excess arterial inflow resulted in 330 ± 37% repayment of the blood flow debt incurred during the preceding occlusion. When, after a 10-second coronary occlusion, the occluder was only partially deflated to provide a residual stenosis which allowed coronary flow to return to the preocclusion rate and was held at this level for 20 or 60 seconds, complete release of the occlusion was followed by an augmented reactive hyperemic response with significant increases in both the volume of excess blood flow and the duration of the reactive hyperemia (Fig. 1; Table 3). Five minutes after administration of nitroglycerin, neither the duration of the reactive hyperemia nor the blood flow debt repayment following a simple 10-second coronary occlusion was different from the control response. Although nitroglycerin did not alter the reactive hyperemia following a simple 10-second coronary artery occlusion, nitroglycerin did reduce reactive hyperemia blood flow when a 10-second occlusion was followed by a 20- or 60-second partial release of the occluder before allowing the reactive hyperemic response to occur (Table 3). The reactive hyperemic response following nitroglycerin was briefer than control when a 10-second occlusion was followed by a 60-second partial release phase (P < 0.05), but not with a 20-second partial release. In contrast to the effects of nitroglycerin, nifedipine significantly reduced the volume of reactive hyperemic blood flow after a simple 10-second coronary occlusion, decreasing the blood flow debt repayment from 330 ± 37% to 175 ± 38% (P < 0.02). In addition, nifedipine prevented augmentation of the reactive hyperemic response that normally occurred when a 10-second occlusion was followed by either a 20- or 60-second interval during which blood flow was restricted to the pre-occlusion control level (Table 3).

Left ventricular myocardial blood flow measured with microspheres in the anterior control region and the posterior region supplied by the left circumflex coronary artery is shown in Table 4 and Figure 2.
During control conditions, there was no significant difference in mean blood flow or in the endo:epi ratio between the anterior and posterior left ventricular wall. Mean blood flow and the transmural distribution of perfusion in the anterior control region were not altered during the partial release phase following a 10-second coronary occlusion (coronary stenosis), nor were they altered by administration of nitroglycerin or nifedipine (Table 4). Although mean blood flow to the posterior left ventricle was not different from control during the partial release phase, the coronary stenosis resulted in transmural redistribution of blood flow away from the subendocardium (endo:epi fell from 1.45 ± 0.06 during control conditions to 0.50 ± 0.04 during the partial release phase; P < 0.01). During the partial release phase, nitroglycerin resulted in significant reduction of blood flow to the subepicardium (layer 1) from 1.27 ± 0.18 to 1.07 ± 0.14 ml/min per g (P < 0.02), while increasing delivery of blood flow to the subendocardium (layer 4) from 0.62 ± 0.08 to 0.77 ± 0.10 ml/min per g of myocardium (P < 0.05). This change in the transmural distribution of myocardial blood flow during the partial release phase following nitroglycerin resulted in an increase of the endo:epi ratio from 0.50 ± 0.04 to 0.85 ± 0.07 (P < 0.01). Similarly, during the partial release phase, nifedipine decreased blood flow to the subepicardium from 1.27 ± 0.18 to 0.90 ± 0.13 ml/min per g (P < 0.02) while simultaneously increasing blood flow to layer 4 from 0.62 ± 0.08 to 0.85 ± 0.09 ml/min per g of myocardium (P < 0.03). Thus, the endo:epi ratio during the partial release phase increased from 0.50 ± 0.04 during control conditions to 1.11 ± 0.23 following nifedipine administration (P < 0.01).

**Group II**

Left circumflex coronary artery blood flow, arterial and coronary sinus oxygen content, coronary arteriovenous oxygen difference, and myocardial oxygen consumption for six dogs in group II during control conditions and 30 min after administration of nifedipine are shown in Table 5. None of these variables was significantly altered 30 minutes after administration of nifedipine.

**Group III**

Regional myocardial blood flow during control conditions and during total occlusion of the left circumflex.
FIGURE 2. Myocardial blood flow to four transmural layers from the posterior left ventricular wall perfused by the left circumflex coronary artery is shown for seven dogs. Measurements were obtained during control conditions with unimpeded inflow and in the presence of a coronary stenosis which prevented blood flow from increasing above the pre-occlusion control level after a 10-second total coronary occlusion (panel A). The effects of nitroglycerin and nifedipine on the transmural distribution of myocardial blood flow in the presence of a coronary stenosis are shown in panels B and C. * indicates P < 0.05 in comparison with blood flow to the corresponding myocardial layer.

The coronary reactive hyperemic response is closely coupled to myocardial metabolic activity during the interval of arterial occlusion so that alterations of myocardial metabolic activity or alterations of the duration of occlusion result in commensurate alterations of the subsequent reactive hyperemia to maintain a nearly constant blood flow debt repayment (Olsson and Gregg, 1965b; Pauly et al., 1973; Bache et al., 1973). Because such a close relationship exists between the degree of myocardial ischemia during the interval of coronary occlusion and the subsequent reactive hyperemø response, the magnitude of the reactive hyperemia may be used to evaluate the degree of preceding ischemia. For this reason, the finding that the reactive hyperemia following a 10-second coronary occlusion was augmented when the occlusion was followed by a 20- or 60-second interval of restricted arterial inflow suggested that myocardial ischemia was continuing during the interval of restricted inflow. Since during the interval of restricted inflow the degree of coronary stenosis was adjusted to maintain blood flow at a level which was adequate to maintain the myocardium prior to the occlusion, it was necessary to explain why this same volume of arterial inflow was not adequate to prevent continuing ischemia during the coronary stenosis.

Previous studies have demonstrated that when a proximal coronary stenosis reduces arterial inflow below myocardial metabolic requirements, the resultant hypoperfusion is not uniform. Rather, transmural

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Left Circumflex Coronary Artery Blood Flow and Myocardial Oxygen Consumption in Six Dogs during Control Conditions and 30 Minutes after Administration of Nifedipine (0.01 mg/kg, iv)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coronary blood flow (ml/min)</strong></td>
<td><strong>Arterial oxygen content (ml/100 ml)</strong></td>
</tr>
<tr>
<td>Control</td>
<td>27.5 ± 5.1</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>29.3 ± 6.0</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

There was no statistically significant difference between any values during control conditions and after nifedipine.
redistribution of perfusion occurs, to result in preferential blood flow to the outer myocardial layers, thereby resulting in most severe hypoperfusion in the subendocardium (Salisbury et al., 1963; Griggs and Nakamura, 1968; Bache et al., 1977). In the present study, however, blood flow during the partial release phase was not reduced, but rather was maintained equal to that observed during the preocclusion period. Nevertheless, when microspheres were injected during the partial release phase, although mean flow was equal to that observed during the control period, transmural redistribution of perfusion had occurred, to result in increased flow to the subepicardium at the expense of continuing hypoperfusion in the subendocardium. This continuing subendocardial underperfusion during the partial release phase explains the augmentation of the reactive hyperemic response which was observed when a 10-second occlusion was followed by an interval of restricted inflow.

Both nitroglycerin and nifedipine prevented augmentation of the reactive hyperemic response which occurred when a 10-second total coronary occlusion was followed by a period of restricted inflow. Following nifedipine administration, this effect could potentially have resulted from the direct effect of nifedipine on depressing coronary reactive hyperemia. Unlike nifedipine, however, nitroglycerin did not directly alter the reactive hyperemia following a 10-second occlusion. Measurements of the transmural distribution of perfusion during the interval of restricted inflow showed that these agents inhibited the marked redistribution of perfusion away from the subendocardium in the presence of a flow-limiting coronary stenosis. By inhibiting this redistribution of perfusion, these agents appeared to decrease the degree of subendocardial ischemia during the partial release phase; this mechanism may have contributed to preventing augmentation of the subsequent reactive hyperemia.

Administration of nifedipine produced transient coronary vasodilation which was followed by depression of the coronary reactive hyperemic response. Several mechanisms which could potentially decrease the reactive hyperemic response were evaluated. Vasodilation of intercoronary collateral channels by nifedipine would increase collateral flow and thereby decrease the degree of ischemia during occlusion. However, direct measurement showed that nifedipine had no effect on collateral flow during total coronary occlusion. This is in agreement with similar findings reported by Weintraub et al. (1981), but is contrary to the reports of Henry et al. (1978) and Clark et al. (1979), who found that nifedipine increased blood flow to ischemic myocardium in dogs with acute occlusion of the left anterior descending coronary artery. These apparent differences in the response of blood flow in ischemic myocardial regions to nifedipine may have resulted from technical differences between these studies. Thus, Henry et al. (1978) and Clark et al. (1979) used larger dosages of nifedipine that resulted in sustained reductions in arterial pressure and sustained increases of blood flow to the normally perfused myocardial areas, whereas, in the present study, aortic pressure and blood flow in normally perfused myocardium had returned to the pretreatment level before measurements were performed. Weintraub et al. (1981) found that the method of tissue sampling may affect the apparent behavior of ischemic zone blood flow. These workers found that when even small peninsulas of normally perfused myocardium were included in the ischemic tissue specimens, nifedipine caused an apparent increase in ischemic zone flow which did not occur when care was taken to exclude all normally perfused tissue. In the present study, injection of the left circumflex coronary artery with methylene blue dye was used to identify the collateral-dependent myocardium, and a 1.0-cm cuff of blue-stained myocardium was maintained around the ischemic zone specimens when the heart was sectioned. This sampling technique was used to eliminate border areas of intermingling normally perfused and collateral dependent myocardium and thereby ensure that only truly collateral-dependent tissue was sampled.

Second, nifedipine could have blunted the reactive
hyperemic response by causing a reduction of myocardial oxygen requirements. Nagao et al. (1980) reported that infusion of the calcium channel blocker diltiazem prior to and during 5- and 10-minute coronary occlusions in open chest dogs resulted in significant reductions of both heart rate and arterial pressure, and decreased the subsequent impairment of left ventricular developed tension and mitochondrial respiration. They suggested that reduction of the load on the heart by diltiazem during occlusion preserved subsequent myocardial activity. However, at the time that the reactive hyperemic response was depressed in the present study, nifedipine had no significant effect on heart rate, blood pressure, or measured myocardial oxygen consumption. The disparity in the results between the report of Nagao et al. (1980) and the present study may be related to the different calcium channel-blocking agent used, different experimental preparations (open chest vs. chronically instrumental awake dogs), different durations of total coronary occlusion (5 or 10 minutes vs. 10 seconds), and different methods of drug administration (continuous infusion vs. bolus injection).

The lack of effect of nifedipine on myocardial oxygen consumption and on collateral blood flow, as well as the absence of significant change in heart rate or arterial pressure during the sequence of studies, implies that the degree of myocardial ischemia during a 10-second coronary occlusion was not altered by nifedipine. Therefore, depression of the reactive hyperemia suggests that nifedipine produced a dissociation between myocardial ischemia and the degree of coronary vasodilation. Since the reactive hyperemic response has been shown to result from vasodilation of the coronary microvasculature without involvement of the epicardial arteries (Cohen and Kirk, 1973), this effect of nifedipine would appear to occur at the level of the coronary resistance vessels. Although potent coronary vasodilators such as adenosine—which increase coronary flow in excess of myocardial metabolic needs—may depress the reactive hyperemic response, coronary flow was not increased at the time the reactive hyperemia was depressed by nifedipine (Bache et al., 1973).

Depression of vasodilation of the coronary resistance vessels in response to a brief ischemic stimulus could have important therapeutic implications. Ischemic vasodilation of the coronary resistance vessels results in loss of the ability for normal autoregulation and decreased perfusion pressure distal to the stenosis (Bache and Cobb, 1977; Bache et al., 1977). In the presence of a proximal coronary stenosis, these effects may combine to result in redistribution of blood flow away from the subendocardium. It is reasonable that nifedipine, by blunting the intense vasodilator response to ischemia, would enhance subendocardial perfusion. Although depression of the vasodilator response of the coronary resistance vessels might have a beneficial effect on subendocardial perfusion, could reduction of reactive hyperemia blood flow impair recovery of the myocardium after a period of ischemia? This is unlikely, since previous studies suggest that the vasodilation which occurs during coronary reactive hyperemia results in blood flow in excess of myocardial requirements. Thus, coronary sinus oxygen content has been shown to increase to far above normal levels during the reactive hyperemic response, suggesting that the coronary vessels are delivering arterial inflow far above myocardial needs (Olsson and Gregg, 1965a). In addition, we have shown that if only the initial portion of the reactive hyperemia is allowed to occur, resulting in approximately 100% blood flow debt repayment, coronary vascular tone may be regained without the usual markedly excess arterial inflow (Bache et al., 1974). These data suggest that the usual coronary reactive hyperemic response results in inflow of far more arterial blood than is actually required by the myocardium.

Unlike nifedipine, nitroglycerin did not depress the vasodilator response of the coronary resistance vessels to a brief ischemic stimulus. Fam and McGregor (1964) showed that nitroglycerin exerts only a brief vasodilator effect on the coronary resistance vessels, but a more sustained effect on the epicardial muscular arteries. In contrast, myocardial ischemia causes vasodilation of the resistance vessels, but not of the epicardial arteries (Winbury et al., 1969; Cohen and Kirk, 1973). Winbury et al. (1969) have provided evidence that the penetrating arteries which conduct blood from the epicardium to the subendocardial muscle also do not participate in metabolic autoregulation and consequently are not maximally vasodilated by the presence of ischemia. When myocardial ischemia results in maximal vasodilation of the coronary resistance vessels, the small amount of resistance offered by the penetrating arteries may assume relatively greater importance. By dilating these penetrating arteries and thereby decreasing the vascular resistance separating the subendocardium from the subepicardium, nitroglycerin or nifedipine could act to facilitate delivery of blood to the subendocardium (Winbury et al., 1969). However, this mechanism is not supported by the failure of nitroglycerin or nifedipine to enhance subendocardial blood flow during total coronary occlusion.

In patients with angina pectoris, both nitroglycerin and nifedipine have been shown to decrease arterial pressure during exercise; the resultant reduction of left ventricular systolic tension would be expected to reduce myocardial oxygen requirements and thereby exert a direct antianginal effect (Atterhog et al., 1975; Robinson, 1978). In addition, the present study demonstrates that both of these agents have the potential for enhancing subendocardial perfusion when arterial inflow is limited by proximal coronary stenosis. This ability to enhance delivery of blood to the subendocardium, where vulnerability to ischemia is greatest, may represent an additional significant therapeutic effect of these agents.

This study was supported by U.S. Public Health Service Grants HL-20598 and HL-21872 from the National Heart, Lung, and Blood Institute.
References


Pauly TJ, Zarnstorff WC, Bittar N (1973) Myocardial metabolic activity as a determinant of reactive hyperemia responses in the dog heart. Cardiovascular Res 7: 90-94


Effect of nitroglycerin and nifedipine on subendocardial perfusion in the presence of a flow-limiting coronary stenosis in the awake dog.
R J Bache and B A Tockman

Circ Res. 1982;50:678-687
doi: 10.1161/01.RES.50.5.678

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/50/5/678.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/