ATP-Sensitive K⁺ Channels, Adenosine, and Nitric Oxide–Mediated Mechanisms Account for Coronary Vasodilation During Exercise

Yutaka Ishibashi, Dirk J. Duncker, Jianyi Zhang, Robert J. Bache

Abstract—We previously reported that combined blockade of adenosine receptors and ATP-sensitive K⁺ channels (K⁺ATP channels) blunted but did not abolish the response of coronary blood flow to exercise. This study tested the hypothesis that the residual increase in coronary flow in response to exercise after adenosine receptor and K⁺ATP channel blockade is dependent on endogenous NO. Dogs were studied at rest and during a four-stage treadmill exercise protocol under control conditions, during K⁺ATP channel blockade with glibenclamide (50 μg · kg⁻¹ · min⁻¹ IC) in the presence of adenosine receptor blockade with 8-phenyltheophylline (8-PT, 5 mg/kg IV), and after the addition of the NO synthase inhibitor N⁶-nitro-L-arginine (LNNA, 1.5 mg/kg IC). During control conditions, coronary blood flow was 49±3 mL/min at rest and increased to 92±8 mL/min at peak exercise. LNNA alone or in combination with 8-PT did not alter resting coronary flow and did not impair the normal increase in flow during exercise, indicating that when K⁺ATP channels are intact, neither NO nor adenosine-dependent mechanisms are obligatory for maintaining coronary blood flow. Combined K⁺ATP channel and adenosine blockade decreased resting coronary flow to 27±3 mL/min (P<.05), but exercise still increased flow to 45±5 mL/min (P<.05). The subsequent addition of LNNA further decreased resting coronary flow to 20±2 mL/min and markedly blunted exercise-induced coronary vasodilation (coronary vascular conductance, 0.20±0.03 mL · min⁻¹ · mm Hg⁻¹ at rest versus 0.24±0.04 mL · min⁻¹ · mm Hg⁻¹ during the heaviest level of exercise; P=.22), so that coronary flow both at rest and during exercise was below the control resting level. The findings suggest that K⁺ATP channels are critical for maintaining coronary vasodilation at rest and during exercise but that when K⁺ATP channels are blocked, both adenosine and NO act to increase coronary blood flow during exercise. In the presence of combined K⁺ATP channel blockade and adenosine receptor blockade, NO was able to produce approximately one quarter of the coronary vasodilation that occurred in response to exercise when all vasodilator systems were intact. (Circ Res. 1998;82:346-359.)

Key Words: blood flow ■ endothelium ■ K⁺ channel ■ ischemia ■ reactive hyperemia

Recent evidence indicates that hyperpolarization of the vascular smooth muscle cell membrane caused by opening of K⁺ATP channels is an important mechanism for metabolic coronary vasodilation.¹ We previously reported that blockade of vascular smooth muscle K⁺ATP channels in chronically instrumented dogs decreased resting coronary blood flow but did not attenuate the increase in flow that occurred in response to treadmill exercise.² However, after K⁺ATP channel blockade had been established, the subsequent addition of adenosine receptor blockade reduced the exercise-induced increase in coronary flow by more than half,³ indicating increased importance of adenosine in causing metabolic vasodilation after K⁺ATP channels are blocked. Nevertheless, even after the combination of K⁺ATP channel blockade and adenosine receptor blockade, exercise still produced a substantial increase in coronary flow.

Bernstein et al⁴ have reported that exercise causes increased NO production across the coronary circulation. We have previously observed that NO contributes to coronary vasodilation distal to a coronary artery stenosis, which results in hypoperfusion during exercise.³ It is possible that NO could similarly contribute to coronary vasodilation when K⁺ATP channel and adenosine receptor blockade have resulted in myocardial hypoperfusion during exercise. We consequently hypothesized that failure of the combination of adenosine receptor and K⁺ATP channel blockade to abolish coronary vasodilation in response to exercise could be accounted for by increased importance of endothelium-derived NO. To test this hypothesis, we examined the effect of NO synthase inhibition after the combination of adenosine receptor and K⁺ATP channel blockade on the increase in coronary blood flow produced by treadmill exercise in chronically instrumented dogs. To assess the hierarchy of importance of these three vasodilator systems, we also examined the effects of NO synthase inhibition alone and in combination with adenosine receptor blockade on exercise-induced coronary vasodilation in the absence of K⁺ATP channel blockade.

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Selected Abbreviations and Acronyms

- 8-PT = 8-phenylephedrine
- K\textsuperscript{+}\textsubscript{ATP} channel = ATP-sensitive K\textsuperscript{+} channel
- K\textsuperscript{+}\textsubscript{Ca\textsuperscript{2+}} channel = Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel
- L-NAME = nitro-arginine methyl ester
- L-NMMA = N-nitro-L-arginine
- LAD = left anterior descending coronary artery
- LNNA = N\textsuperscript{5}-nitro-arginine
- LV = left ventricular

Materials and Methods

Studies were performed in 31 adult mongol dogs weighing 20 to 27 kg and trained to run on a motor-driven treadmill. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 93–23, revised 1985) as approved by the Council of the American Physiological Society and with prior approval of the Animal Care Committee of the University of Minnesota.

Surgical Preparation

After sedation with acepromazine (0.5 mg/kg IM), dogs were anesthetized with sodium pentobarbital (30 to 35 mg/kg IV), intubated, and ventilated with a mixture of oxygen (30%) and room air (70%) to maintain arterial blood gases within the physiological range. A left thoracotomy was performed in the fifth intercostal space, and a polyvinyl chloride catheter (outer diameter, 3.0 mm) filled with heparinized saline was inserted into the internal thoracic artery and advanced into the ascending aorta. The heart was suspended in a pericardial cradle, and catheters were introduced into the left atrium through the atrial appendage and into the left ventricle through the apical dimple. A solid-state micromanometer (model 5S, Konigsberg Instrument Co) was also introduced into the left ventricle at the apex. A final catheter was introduced into the right atrial appendage, manipulated into the coronary sinus ostium, and advanced into the great cardiac vein until the tip could be palpitated within 1 cm of the interventricular sulcus to allow selective sampling of coronary venous blood draining the myocardium perfused by the LAD. Approximately 1.5 cm of the proximal LAD was dissected free, and a Doppler velocity probe (Craig Hartley) was positioned around the artery. Immediately distal to the velocity probe, a hydraulic occluder was placed around the vessel. A silicone catheter (inner diameter, 0.3 mm) bonded to a larger silicone catheter (inner diameter, 1.6 mm) was introduced into the LAD immediately distal to the hydraulic occluder. Miniature 10-MHz Doppler crystals for measurement of myocardial wall thickening were sutured onto the epicardium in the regions perfused by the LAD and the left circumflex coronary artery (control region), respectively. The pericardium was then loosely closed, and the catheters and electrical leads were tunneled subcutaneously to exit at the base of the neck. The chest was closed in layers, and the pneumothorax was evacuated. Catheters were flushed daily with heparinized saline.

Heamodynamic Measurements

Studies were performed 2 to 3 weeks after surgery with the animal either resting quietly in a sling or exercising on a motor-driven treadmill. Phasic and mean aortic pressure was measured with a Gould P23XL pressure transducer positioned at midst level. LV pressure was measured with the micromanometer calibrated with the fluid-filled LV catheter. LV dP/dt was obtained via electrical differentiation of the LV pressure signal. Coronary blood flow velocity was measured with a Doppler flowmeter system (Craig Hartley). Data were recorded on an eight-channel direct-writing oscillograph (Coulbourn Instruments).

Myocardial Oxygen Consumption

In 16 dogs, myocardial oxygen consumption was measured at rest and during exercise. Blood specimens were maintained in iced syringes until completion of each exercise study. Measurements of \text{P\textsubscript{O2}}, \text{P\textsubscript{CO2}}, and \text{pH} were then immediately performed with an Instrumentation Laboratory model 113 blood gas analyzer. Hemoglobin content was...
determined with the cyanmethemoglobin method. Hemoglobin oxygen saturation was determined from the blood PO2, pH, and temperature by using the oxygen dissociation curve for canine blood. Blood oxygen content was computed as follows: (hemoglobin × 1.34 × %O2 saturation) + (0.0031 × PO2). Oxygen consumption in the region of myocardium perfused by the LAD was calculated as the product of blood flow determined from the Doppler flow velocity probe (see “Data Analysis”) and the difference in oxygen content between aortic and coronary venous blood.

Regional Myocardial Function Measurements
Regional myocardial wall thickening was measured using single epicardial 10-MHz ultrasonic pulsed Doppler crystals (Craig Hartley). The onset of systole was taken as the initiation of the upstroke of LV pressure recorded from the micromanometer; end systole was taken 20 milliseconds before peak negative dP/dt. Maximum systolic excursion (SE) was recorded at a range-gate depth (D) of 9 mm. Percent myocardial systolic wall thickening (%WT) was calculated as follows: %WT = SE/D.

Experimental Protocols

Efficacy of Pharmacological Antagonists

Degree of Adenosine Receptor Blockade by 8-PT
In 7 resting dogs, the magnitude and selectivity of adenosine receptor blockade produced by 8-PT was determined. For this purpose, we

Table 1. Reactive Hyperemia After LAD Occlusions of 5-, 10-, 20-, and 30-Second Duration

<table>
<thead>
<tr>
<th>Occlusion Duration</th>
<th>5 s</th>
<th>10 s</th>
<th>20 s</th>
<th>30 s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con</td>
<td>P+G</td>
<td>P+G+L</td>
<td>Con</td>
</tr>
<tr>
<td>Occlusion time, s</td>
<td>5.5±0.2</td>
<td>5.3±0.1</td>
<td>5.3±0.1</td>
<td>10.4±0.3</td>
</tr>
<tr>
<td>Basal flow, mL/min</td>
<td>43±6</td>
<td>32±8*</td>
<td>19±5†</td>
<td>45±6</td>
</tr>
<tr>
<td>Flow debt, mL</td>
<td>5±1</td>
<td>4±1*</td>
<td>2±1*</td>
<td>8±1</td>
</tr>
<tr>
<td>Peak flow, mL/min</td>
<td>124±11</td>
<td>74±11*</td>
<td>47±6†</td>
<td>143±8</td>
</tr>
<tr>
<td>RH duration, s</td>
<td>25±3</td>
<td>14±2</td>
<td>8±2†</td>
<td>35±6</td>
</tr>
<tr>
<td>RH excess flow, mL</td>
<td>10±2</td>
<td>5±2</td>
<td>2±1†</td>
<td>17±7</td>
</tr>
<tr>
<td>RH repayment, %</td>
<td>210±18</td>
<td>122±16</td>
<td>92±10†</td>
<td>211±27</td>
</tr>
</tbody>
</table>

Con indicates control; P, 5 mg/kg IV 8-PT; G, 50 μg·kg⁻¹·min⁻¹ IC glibenclamide; L, 1.5 mg/kg IC LNNA; and RH, reactive hyperemia. Values are mean±SEM (n=6). *P<.05 vs corresponding Con measurements; †P<.05 vs corresponding P+G measurements.
measured the increases in coronary blood flow caused by intracoronal adenosine (0.5 to 25 μg·kg⁻¹·min⁻¹), the K⁺,ATP channel opener pinacidil (0.2 to 2.5 μg·kg⁻¹·min⁻¹), and sodium nitroprusside (0.3 to 3 μg·kg⁻¹·min⁻¹), infused in random order. After completion of these measurements, adenosine receptor blockade was produced with 8-PT (5 mg/kg) infused intravenously over 10 minutes. Ten minutes after completion of drug administration, intracoronary infusions of adenosine (2 to 25 μg·kg⁻¹·min⁻¹), pinacidil (0.2 to 5 μg·kg⁻¹·min⁻¹), and nitroprusside (0.3 to 3 μg·kg⁻¹·min⁻¹), and sodium nitroprusside (0.3 to 2 μg·kg⁻¹·min⁻¹) infused in random order. After completion of these measurements, adenosine receptor blockade was produced with 8-PT (5 mg/kg) infused intravenously over 10 minutes. Ten minutes after completion of drug administration, intracoronary infusions of adenosine (2 to 25 μg·kg⁻¹·min⁻¹) were repeated while coronary blood flow responses were recorded.

**Exercise Protocol**

**Group 1**

On a different day, 12 dogs underwent graded treadmill exercise. With the dogs standing quietly on the treadmill, resting hemodynamics and wall thickening measurements were obtained, and arterial and coronary venous blood samples were collected. Subsequently, a four-stage treadmill exercise protocol was begun (4.8 km/h at 0% grade, 6.4 km/h at 5% grade, and 6.4 km/h at 10% grade). Each exercise stage was 3 minutes in duration. LV and aortic pressures, coronary blood flow, and systolic wall thickening were measured, and blood samples were collected during the last 30 seconds of each exercise stage.

After 90 minutes of rest, adenosine receptor blockade was produced with 8-PT (5 mg/kg IV). Ten minutes later, an intracoronary infusion of glibenclamide (50 μg·kg⁻¹·min⁻¹) was started. Five minutes after beginning the glibenclamide infusion, the four-stage exercise protocol was repeated during glibenclamide receptor blockade and glibenclamide infusion. After completion of exercise, the glibenclamide infusion was discontinued, and the dogs were allowed to rest for 90 minutes. Then 8-PT was again administered in a dose of 2.5 mg/kg IV. Ten minutes later, LNNA was infused into the coronary artery in a dose of 1.5 mg/kg over 15 minutes at a rate of 0.6 mL/min. Ten minutes after completion of the LNNA infusion, the intracoronary glibenclamide infusion was restarted (50 μg·kg⁻¹·min⁻¹). Five minutes after beginning the glibenclamide infusion, the exercise protocol was repeated during combined adenosine receptor blockade, NO synthase inhibition, and K⁺,ATP channel blockade.

**Data Analysis**

Heart rate, LV and aortic pressures, coronary blood flow, and regional wall thickening were measured from the strip-chart recordings. Coronary blood flow was computed from the Doppler shift using the equation $Q = \frac{2.5 \times f \times D^2}{\pi \times c \times \cos \theta}$, where $Q$ is the coronary flow (mL/min), $f$ is the Doppler shift (kHz), and $D$ is the internal diameter of the coronary artery. The factor 2.5 is a constant derived from the speed of sound in tissue ($c = 1.5 \times 10^5$ cm/s), the frequency of the emitted sound beam ($f_0 = 40$ kHz), and the cosine of the angle at which the sound beam is emitted (45°), and unit conversion factors: $(c \times \pi \times 4 \times 3)/(2 \times c \times \cos \theta)$. 

**Effects of Restoring Coronary Blood Flow**

To determine whether combined adenosine blockade and NO blockade inhibits coronary vasodilation during exercise, the four-stage treadmill exercise protocol was repeated during drug administration, intracoronary infusion of LNNA (1.5 mg/kg), and after LNNA combined with 8-PT (5 mg/kg IV) in 6 dogs. Dogs were allowed to rest for 90 minutes between exercise trials.

Effects of restoring coronary blood flow to the control pretreatment level was examined in 5 dogs. The study was performed while dogs were standing quietly in a sling.

**Inhibition of NO Production by LNNA**

The magnitude and selectivity of glibenclamide as a K⁺,ATP channel blocker was assessed in 5 resting dogs. For this purpose, we measured the increases in coronary blood flow produced by the K⁺,ATP channel opener pinacidil (0.25 to 2.5 μg·kg⁻¹·min⁻¹) infused into the coronary artery catheter. After washout of pinacidil, an intracoronary infusion of glibenclamide at a dose of 50 μg·kg⁻¹·min⁻¹ was started. Five minutes later, while the glibenclamide infusion was continued, the pinacidil infusions were repeated (0.5 to 5 μg·kg⁻¹·min⁻¹). On separate days, we studied the effects of glibenclamide on the increases in coronary blood flow produced by nitroprusside (0.6 to 6 μg·kg⁻¹·min⁻¹, n=4) and adenosine (1 to 50 μg·kg⁻¹·min⁻¹, n=5).

Inhibition of NO Production by LNNA

The magnitude and selectivity of glibenclamide as a NO synthase inhibitor was assessed in 7 resting dogs. For this purpose, we measured the increases in coronary blood flow produced by intracoronary acetylcholine (0.5 to 4 mg·kg⁻¹·min⁻¹) and nitroprusside (0.3 to 3 μg·kg⁻¹·min⁻¹) infused into the coronary artery catheter. After washout of acetylcholine, an intracoronary infusion of glibenclamide at a dose of 50 μg·kg⁻¹·min⁻¹ was started. Five minutes later, while the glibenclamide infusion was continued, the acetylcholine infusions were repeated (0.5 to 4 mg·kg⁻¹·min⁻¹) and nitroprusside (0.3 to 3 μg·kg⁻¹·min⁻¹) were repeated.

**Reactive Hyperemia**

The effects of glibenclamide combined with 8-PT on the reactive hyperemic responses to brief coronary artery occlusions were studied with and without NO synthase inhibition in 6 dogs. With dogs resting quietly in a sling, baseline measurements of systemic hemodynamics and coronary blood flow were performed. Then reactive hyperemic responses to coronary occlusions 5, 10, 20, and 30 seconds in duration were recorded in duplicate. A 3-minute interval was allowed between occlusions. Subsequently, 8-PT was administered intravenously in a dose of 5 mg/kg over 10 minutes. Ten minutes after completion of drug administration, intracoronary infusion of glibenclamide in a dose of 50 μg·kg⁻¹·min⁻¹ was started into the coronary artery at a rate of 1.5 mL/min. Five minutes later, hemodynamic measurements were collected, and the reactive hyperemic responses to coronary artery occlusions 5, 10, 20, and 30 seconds in duration were obtained in the presence of 8-PT and glibenclamide. The glibenclamide infusion was then discontinued, and NO synthase inhibition was produced with LNNA at a dose of 1.5 mg/kg IC over 15 minutes at a rate of 0.6 mL/min. Ten minutes after completion of LNNA, the glibenclamide infusion was restarted at a dose of 50 μg·kg⁻¹·min⁻¹. Five minutes later, hemodynamic measurements were obtained, and the reactive hyperemic responses to coronary artery occlusions 5, 10, 20, and 30 seconds in duration were repeated in the presence of combined adenosine receptor blockade, NO synthase inhibition, and K⁺,ATP channel blockade.

**Group 2**

To determine whether combined adenosine blockade and NO blockade inhibits coronary vasodilation during exercise, the four-stage treadmill exercise protocol was repeated during drug administration, intracoronary infusion of LNNA (1.5 mg/kg), and after LNNA combined with 8-PT (5 mg/kg IV) in 6 dogs. Dogs were allowed to rest for 90 minutes between exercise trials.

**Effects of Restoring Coronary Blood Flow**

To determine whether the decrease in systolic wall thickening and myocardial oxygen consumption after the combination of glibenclamide and 8-PT with LNNA resulted from impaired myocardial perfusion, the effect of restoring coronary blood flow to the control pretreatment level was examined in 5 dogs. The study was performed while dogs were standing quietly in a sling.

**Hemodynamics, coronary blood flow, and systolic wall thickening were obtained during basal conditions and during intracoronary infusion of nitroprusside (1.5 μg·kg⁻¹·min⁻¹) infused rate, 0.15 mL/min). Measurements were repeated during intracoronary infusion of glibenclamide (50 μg·kg⁻¹·min⁻¹ IC) combined with 8-PT (5 mg/kg IV) and after the addition of LNNA (1.5 mg/kg IC over 15 minutes). Five minutes after the infusion of drugs, intracoronary infusion of nitroprusside (1.5 μg·kg⁻¹·min⁻¹) was started to restore coronary blood flow to the control pretreatment level. After 5 minutes of infusion of nitroprusside, hemodynamics, coronary blood flow, and systolic wall thickening were again measured.
Coronary Blood Flow During Exercise

Figure 5. Analog recordings of hemodynamic data in an individual animal at rest and during the heaviest level of exercise (stage 4).

TABLE 2. Systemic Hemodynamic Data at Rest and During Graded Treadmill Exercise in Group 1

<table>
<thead>
<tr>
<th>Exercise Level</th>
<th>Rest</th>
<th>P 1&lt;sup&gt;G&lt;/sup&gt;</th>
<th>P 1&lt;sup&gt;G&lt;/sup&gt;+L</th>
<th>P 2&lt;sup&gt;G&lt;/sup&gt;</th>
<th>P 2&lt;sup&gt;G&lt;/sup&gt;+L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (bpm)</td>
<td>164±6</td>
<td>166±7</td>
<td>170±8</td>
<td>172±6</td>
<td>174±7</td>
</tr>
<tr>
<td>Mean Aortic Pressure (mm Hg)</td>
<td>94±2</td>
<td>96±3</td>
<td>100±3</td>
<td>102±3</td>
<td>94±2</td>
</tr>
<tr>
<td>LV Systolic Pressure (mm Hg)</td>
<td>136±3</td>
<td>138±4</td>
<td>140±5</td>
<td>142±4</td>
<td>136±3</td>
</tr>
<tr>
<td>LV End-diastolic Pressure (mm Hg)</td>
<td>119±2</td>
<td>120±3</td>
<td>122±3</td>
<td>121±2</td>
<td>119±2</td>
</tr>
<tr>
<td>End-diastolic Volume (mL)</td>
<td>8±1</td>
<td>8±1</td>
<td>8±1</td>
<td>8±1</td>
<td>8±1</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>71±3</td>
<td>72±3</td>
<td>72±3</td>
<td>71±3</td>
<td>71±3</td>
</tr>
<tr>
<td>Stroke Volume (mL)</td>
<td>5±1</td>
<td>5±1</td>
<td>5±1</td>
<td>5±1</td>
<td>5±1</td>
</tr>
</tbody>
</table>

Drugs

- 8-PT: 8-phenyltheophylline (8 mg/kg IV 8-PT)
- G: 50 µg/kg IV glibenclamide (G50)
- L: 1.5 mg/kg IC LNNA

Total blood flow during reactive hyperemia was determined by electrical integration of the Doppler shift tracing. Reactive hyperemia (mL) was calculated as follows: blood flow debt (mL) = total flow during reactive hyperemia (mL) - control blood flow (mL). The duration of reactive hyperemia was taken as the time from release of coronary occlusion to the point at which flow returned to within 5% of control.

Coronary Blood Flow During Exercise

In chronically instrumented animals, the flow velocity probe is tightly adherent to the coronary artery, so that the internal diameter of the coronary artery effects control and intervention conditions equally. Coronary vascular conductance was calculated by dividing mean coronary blood flow by mean aortic pressure.

Statistical comparisons of hemodynamic measurements, coronary blood flow, and myocardial function were performed using two-way (exercise level and treatment) ANOVA for repeated measures. When a significant effect of treatment was observed, comparisons within groups were performed using one-way ANOVA followed by Scheffé's test. The effect of treatment on the relation between two variables was analyzed by ANCOVA. Statistical significance was accepted at P<0.05. All data are presented as mean±SEM.

**Notes:**
- *P<0.05 vs rest; †P<0.05 vs corresponding Con measurements; ‡P<0.05 vs corresponding P 1<sup>G</sup> measurements.
Results

Efficacy of Pharmacological Antagonists

Degree of Adenosine Receptor Blockade by 8-PT
During resting conditions, intracoronary infusions of adenosine had no effect on heart rate (116 ± 10 bpm) or mean aortic pressure (84 ± 6 mm Hg). Infusion of the KATP opener pinacidil caused a small increase in heart rate from 117 ± 11 bpm during control conditions to 122 ± 11 bpm at the highest dose (P < 0.05), with no significant effect on aortic pressure. Sodium nitroprusside decreased mean aortic pressure from 85 ± 5 to 80 ± 5 mm Hg (P < 0.05) and increased heart rate from 124 ± 8 to 130 ± 10 bpm (P < 0.05). 8-PT had no effect on mean aortic pressure or heart rate and did not alter the systemic responses to adenosine, pinacidil, or nitroprusside. The increases in coronary flow produced by intracoronary infusions of adenosine were markedly attenuated by 8-PT with 82 ± 6% inhibition of the increase in coronary flow produced by adenosine in a dose of 10 μg · kg⁻¹ · min⁻¹, but the coronary flow responses to pinacidil and nitroprusside were not altered (Fig 1). It should be noted that the degree of adenosine blockade was likely underestimated, since the lower blood flow rates during adenosine infusion after 8-PT resulted in less dilution of adenosine and thus higher coronary blood concentrations. Ninety minutes after administration of 8-PT, the adenosine blockade had partially subsided (55 ± 6% inhibition of the increase in coronary flow produced by adenosine, 10 μg · kg⁻¹ · min⁻¹); at this time the addition of 8-PT in a dose of 2.5 mg/kg restored adenosine blockade (83 ± 5% attenuation of the increase in coronary flow produced by adenosine, 10 μg · kg⁻¹ · min⁻¹).

Degree of KATP Channel Blockade by Glibenclamide
Glibenclamide did not significantly alter the systemic hemodynamic responses to adenosine or sodium nitroprusside but abolished the slight increase in heart rate produced by pinacidil during control conditions. Glibenclamide had no effect on the increase in coronary blood flow produced by nitroprusside but markedly decreased the coronary vasodilation caused by pinacidil with 83 ± 5% inhibition of the response to pinacidil at a dose of 2.5 μg · kg⁻¹ · min⁻¹ (Fig 2). The coronary blood flow responses to adenosine were also significantly attenuated; the degree of inhibition of the response to adenosine, 10 μg · kg⁻¹ · min⁻¹, was similar for 8-PT (82 ± 6%) and glibenclamide (81 ± 6%).

Degree of NO Inhibition by LNNA
Intracoronary LNNA (1.5 mg/kg) had no effect on mean aortic blood pressure (90 ± 5 mm Hg) or heart rate (121 ± 7 bpm). Intracoronary infusions of acetylcholine in doses of 0.1 to 4 μg · kg⁻¹ · min⁻¹ had no effect on blood pressure (92 ± 6 mm Hg) or heart rate (118 ± 9 bpm). Coronary blood flow increased from 47 ± 5 mL/min at baseline to 143 ± 15 mL/min during infusion of acetylcholine at a dose of 4 μg · kg⁻¹ · min⁻¹ (Fig 3). After LNNA, this dose of acetylcholine increased coronary flow from 44 ± 5 mL/min at baseline to 74 ± 7 mL/min, representing 69 ± 7% inhibition of the response to acetylcholine. This is likely an underestimate of the degree of blockade, since the decreased coronary flow rates during acetylcholine infusion after LNNA would result in higher blood concentrations than during the control infusion. Intracoronary infusion of nitroprusside in doses of 0.3 to 3 μg · kg⁻¹ · min⁻¹ decreased mean blood pressure from 96 ± 9 mm Hg at baseline to 86 ± 7 mm Hg during the highest dose (P < 0.05), with a tendency for heart rate to increase from 113 ± 10 to 128 ± 14 bpm (P = NS). After LNNA administration, nitroprusside infusion caused similar changes in mean aortic pressure and heart rate. The responses of coronary flow to nitroprusside were not altered by LNNA (Fig 3).

Reactive Hyperemia
During control conditions, heart rate and mean aortic pressure were 115 ± 10 bpm and 88 ± 6 mm Hg, respectively. These values were not significantly altered by 8-PT combined with intracoronary glibenclamide. The addition of LNNA to 8-PT and glibenclamide caused a slight increase in mean aortic blood pressure to 97 ± 4 mm Hg (P < 0.05) with no change in heart rate (120 ± 10 bpm). An example of a reactive hyperemic response to a 20-second coronary artery occlusion is shown in Fig 4. The combination of 8-PT and glibenclamide decreased basal coronary blood flow, decreased peak blood flow during reactive hyperemia, and shortened the duration of reactive hyperemia after 5, 10, 20, and 30-second coronary occlusions. As a result, reactive hyperemia excess flow was markedly attenuated by the combination of 8-PT and glibenclamide (Table 1). The addition of LNNA to the combination of 8-PT and glibenclamide further decreased basal coronary blood flow, decreased peak blood flow during reactive hyperemia, shortened the duration of reactive hyperemia, and decreased reactive hyperemia excess flow (Table 1).

Exercise

Group 1

Systemic Hemodynamics
The hemodynamic responses to increasing levels of exercise are shown in Table 2; data recorded from a representative dog are shown in Fig 5. Exercise caused significant increases in heart rate, mean aortic pressure, LV systolic pressure, LV end-diastolic pressure, and maximal LV dP/dt. The combination of 8-PT and glibenclamide caused significant increases of heart rate and LV end-diastolic pressure at rest but had no effect on any other systemic hemodynamic variable. During exercise, the combination of the two drugs did not alter heart rate or mean aortic pressure. However, the combination of 8-PT and


Table 3. Coronary Hemodynamic Data at Rest and During Graded Treadmill Exercise in Group 1

<table>
<thead>
<tr>
<th>Coronary Blood Flow, mL/min</th>
<th>Coronary Venous Po2, mm Hg</th>
<th>Coronary Venous pH</th>
<th>Coronary Venous Oxygen Tension, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con</td>
<td>P+G</td>
<td>P+G+L</td>
</tr>
<tr>
<td>Rest</td>
<td>49±3</td>
<td>27±3</td>
<td>20±2†</td>
</tr>
<tr>
<td>Exercise (speed, grade)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.8 km/h, 0%</td>
<td>71±6*</td>
<td>32±4</td>
<td>21±3†</td>
</tr>
<tr>
<td>6.4 km/h, 0%</td>
<td>78±7*</td>
<td>34±5</td>
<td>22±4†</td>
</tr>
<tr>
<td>6.4 km/h, 5%</td>
<td>84±7*</td>
<td>39±4†</td>
<td>26±4†</td>
</tr>
<tr>
<td>6.4 km/h, 10%</td>
<td>92±8*</td>
<td>45±5†</td>
<td>29±5†</td>
</tr>
</tbody>
</table>

Con indicates control; P, 5 mg/kg IV 8-PT; G, 50 μg · kg⁻¹ · min⁻¹ IC glibenclamide; and L, 1.5 mg/kg IC LNNA. Values are mean±SEM (n=10).

*P<.05 vs rest; †P<.05 vs corresponding Con measurements; ‡P<.05 vs corresponding P+G measurements.

glibenclamide caused decreases in LV systolic pressure and LV dP/dtmax, and significant elevations of LV end-diastolic pressure. During resting conditions, the addition of LNNA caused a decrease in LV dP/dtmax (P<.05) but had no effect on the other variables. During exercise, the addition of LNNA significantly decreased LV systolic pressure and LVEDP/dtmax compared with the combination of 8-PT and glibenclamide. LV end-diastolic pressure tended to be increased by the addition of LNNA, although this achieved statistical significance only during the first level of exercise (Table 2).

**Coronary Hemodynamics**

As shown in Table 3, myocardial oxygen consumption in the region perfused by the LAD increased from 4.3±0.4 mL/min at rest to 10.5±0.9 mL/min during the highest level of exercise (P<.01). The increase in oxygen consumption was accounted for by an increase in coronary flow from 49±3 mL/min at rest to 92±8 mL/min during the highest level of exercise (P<.01), an increase in hemoglobin from 9.5±0.4 g/dL at rest to 10.8±0.4 g/dL (P<.05), and an increase in the coronary arteriovenous oxygen content difference from 9.4±0.4 mL/dL at rest to 12.0±0.4 mL/dL (P<.01). The increase in myocardial oxygen extraction from 77±2% at rest to 86±1% during the highest level of exercise (P<.01) caused a decrease in coronary venous oxygen tension from 20±1 to 14±1 mm Hg (P<.01).

When 8-PT was combined with glibenclamide, coronary blood flow was significantly lower at rest and during each level of exercise (Table 3), resulting in a downward shift in the slope of the relation between coronary blood flow and heart rate (41.9±7.2×10⁻² mL/beat during control versus 23.7±5.1×10⁻² mL/beat during 8-PT combined with glibenclamide, P<.05) as well as the rate-pressure product (19.2±6.3×10⁻³ mL/beat · mm Hg versus 11.2±2.8×10⁻⁴ mL/beat · mm Hg, P<.01) (Fig 6). The decrease in coronary blood flow was accompanied by a widening of the coronary arteriovenous oxygen content difference and an increase in oxygen extraction (Table 3). Infusion of glibenclamide in the presence of adenosine receptor blockade caused a downward shift of the relationship between coronary venous oxygen tension and myocardial oxygen consumption (P<.05), indicating impairment of the myocardial oxygen supply (Fig 7).

The addition of LNNA significantly further decreased coronary blood flow at rest and during each level of exercise compared with combined 8-PT and glibenclamide (Table 3 and Fig 6). After the addition of LNNA, coronary blood flow underwent a borderline significant increase from 20±2 mL/min at rest to 29±5 mL/min during the highest level of exercise (P=.073). This small residual increase in coronary blood flow in response to exercise resulted principally from an increase in mean aortic pressure (P<.05) without a significant change in coronary vascular conductance (P=.22) (Fig 6).

The slopes of the relation between coronary blood flow and both heart rate and rate-pressure product were further decreased by the addition of LNNA (12.4±4.1×10⁻² mL/beat and 5.2±1.7×10⁻⁴ mL/beat · mm Hg; P<.05 and P<.03, respectively) compared with combined adenosine receptor and K⁺ATP channel blockade (23.7±5.1×10⁻² mL/beat and 11.2±2.8×10⁻⁴ mL/beat · mm Hg) (Fig 5), although the slopes of both relationships remained significantly different from zero (P<.05). Myocardial oxygen extraction was further increased with the addition of LNNA to combined 8-PT and glibenclamide (Table 3), so that for any level of oxygen consumption coronary venous oxygen tension was further reduced (P<.05) (Fig 6).

**Regional Myocardial Contractile Function**

During control conditions, LV transmural systolic wall thickening increased from 21±2% at rest to 30±3% (P<.01) during the highest level of exercise in the LAD-perfused region and from 20±3% to 29±4% (P<.01) in the posterior control region (Fig 8). Combined 8-PT and glibenclamide caused significant decreases in systolic wall thickening at rest and during each level of exercise compared with control conditions, so that systolic wall thickening was decreased to 3±0.3% during the highest level of exercise (P<.01). Systolic wall thickening in the posterior control region was not altered by the LAD infusion of 8-PT and glibenclamide. The addition of LNNA to 8-PT plus glibenclamide caused a slight further decrease in systolic wall thickening in the LAD-perfused region, which was significant under resting conditions. The addition of LNNA also tended to reduce systolic wall thickening in the posterior control region, but this failed to reach statistical significance.

**Group 2**

**Systemic Hemodynamics**

The systemic hemodynamic responses to graded treadmill exercise during control conditions, with LNNA, and with LNNA plus 8-PT are shown in Table 4. LNNA tended to increase heart rate as well as mean aortic and LV systolic pressures both at rest and during exercise, but these changes did
not reach statistical significance. The addition of 8-PT tended to further increase mean aortic and LV systolic pressure during exercise, but this was not significantly different from LNNA alone.

**Coronary Hemodynamics**

As shown in Table 5, during control conditions coronary blood flow increased from 46±5 mL/min at rest to 93±6 mL/min during the heaviest level of exercise (P<.01). Myocardial oxygen consumption increased from 5.9±0.6 mL/min at rest to 12.7±0.6 mL/min during peak exercise (P<.01), and this was associated with a significant decrease in coronary venous oxygen tension. LNNA tended to increase coronary blood flow and decrease coronary venous oxygen tension both at rest and during exercise compared with control, resulting in a significant increase in myocardial oxygen consumption during the two heaviest levels of exercise. The addition of 8-PT to LNNA had no effect on coronary hemodynamics. The relationships between rate-pressure product and coronary blood flow and between myocardial oxygen consumption and coronary venous oxygen tension (Fig 9) were not altered by either LNNA alone or LNNA combined with 8-PT.

**Effects of Increasing Coronary Blood Flow**

As shown in Table 6 and Fig 10, during control conditions intracoronary infusion of sodium nitroprusside (1.5 μg · kg⁻¹ · min⁻¹) increased coronary blood flow with no change of systolic wall thickening or myocardial oxygen consumption. Glibenclamide plus 8-PT decreased coronary blood flow, systolic wall thickening, and myocardial oxygen consumption, with further significant decreases after the addition of LNNA. Restoring coronary blood flow to the baseline level by infusion of nitroprusside caused normalization of regional myocardial function and myocardial oxygen consumption. These findings indicate that the decrease of regional contractile function produced by 8-PT and glibenclamide with or without LNNA resulted from the reduction of coronary blood flow and myocardial oxygen availability produced by these agents.
TABLE 4. Systemic Hemodynamic Data at Rest and During Graded Treadmill Exercise in Group 2

<table>
<thead>
<tr>
<th>Coronary Blood Flow, ml/min</th>
<th>Con</th>
<th>LNNA</th>
<th>L+8-PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>46±5</td>
<td>53±6</td>
<td>51±5</td>
</tr>
<tr>
<td>Exercise (speed, grade)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.8 km/h, 0%</td>
<td>69±9</td>
<td>72±8</td>
<td>72±8</td>
</tr>
<tr>
<td>6.4 km/h, 0%</td>
<td>77±9</td>
<td>82±5</td>
<td>82±5</td>
</tr>
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<td>6.4 km/h, 5%</td>
<td>82±5</td>
<td>91±6</td>
<td>89±7</td>
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<tr>
<td>6.4 km/h, 10%</td>
<td>93±6</td>
<td>106±6</td>
<td>106±6</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Coronary Venous Pco2, mm Hg</th>
<th>Con</th>
<th>LNNA</th>
<th>L+8-PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>36±1</td>
<td>34±0</td>
<td>33±1</td>
</tr>
<tr>
<td>Exercise (speed, grade)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.8 km/h, 0%</td>
<td>33±6</td>
<td>36±0</td>
<td>31±1</td>
</tr>
<tr>
<td>6.4 km/h, 0%</td>
<td>33±0</td>
<td>31±0</td>
<td>30±1</td>
</tr>
<tr>
<td>6.4 km/h, 5%</td>
<td>30±5</td>
<td>29±1</td>
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<tr>
<td>6.4 km/h, 10%</td>
<td>28±9</td>
<td>29±1</td>
<td>27±1</td>
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</table>

<table>
<thead>
<tr>
<th>Coronary Venous pH</th>
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<th>LNNA</th>
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<tbody>
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<td>Rest</td>
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<td>7.4±0.01</td>
<td>7.4±0.02</td>
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<tr>
<td>Exercise (speed, grade)</td>
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<td></td>
<td></td>
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<tr>
<td>4.8 km/h, 0%</td>
<td>7.4±0.01</td>
<td>7.4±0.01</td>
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</tr>
<tr>
<td>6.4 km/h, 0%</td>
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<tr>
<td>6.4 km/h, 5%</td>
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<td>6.4 km/h, 10%</td>
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</table>

<table>
<thead>
<tr>
<th>Coronary Venous Oxygen Tension, mm Hg</th>
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<th>LNNA</th>
<th>L+8-PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>18±1</td>
<td>16±2</td>
<td>15±1</td>
</tr>
<tr>
<td>Exercise (speed, grade)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4.8 km/h, 0%</td>
<td>14±1</td>
<td>12±1</td>
<td>13±1†</td>
</tr>
<tr>
<td>6.4 km/h, 0%</td>
<td>13±1</td>
<td>11±1</td>
<td>11±1†</td>
</tr>
<tr>
<td>6.4 km/h, 5%</td>
<td>12±1</td>
<td>9±1</td>
<td>10±1†</td>
</tr>
<tr>
<td>6.4 km/h, 10%</td>
<td>11±2</td>
<td>8±1</td>
<td>7±1††</td>
</tr>
</tbody>
</table>

Con indicates control; LNNA, 1.5 mg/kg IV LNNA; and L+8-PT, LNNA plus 5 mg/kg IV 8-PT. Values are mean±SEM (n=5).

Con indicates control; LNNA, 1.5 mg/kg IC LNNA; and L+8-PT, LNNA plus 5 mg/kg LV 8-PT. Values are mean±SEM (n=6).

*P<.05 vs rest. †P<.05 vs corresponding Con measurements.

Discussion

The present study identifies the principal mechanisms that account for coronary vasodilation during exercise. Previously, we reported that adenosine receptor blockade had no effect on coronary blood flow at rest or during exercise and that K$_{ATP}$ channel blockade decreased resting coronary blood flow but did not attenuate the increase in coronary flow that occurred in response to exercise. In the presence of K$_{ATP}$ channel blockade, the addition of adenosine receptor blockade did not further decrease resting coronary flow but attenuated the increase in flow produced by exercise by approximately half, suggesting that the coronary constriction that followed K$_{ATP}$ channel blockade resulted in augmented myocardial adenosine production during exercise, which caused coronary vasodilation. In the present study, the addition of NO synthase inhibition, which under normal conditions tended to increase coronary flow at rest and during exercise, caused a further decrease in resting coronary blood flow and nearly abolished coronary vasodilation in response to exercise. The findings imply that K$_{ATP}$ channels are of critical importance for maintaining coronary vasodilation at rest and during exercise but that when the K$_{ATP}$ channels are blocked, increased production of adenosine and NO mediates coronary vasodilation in response to exercise.

Coronary Blood Flow at Rest

In vivo studies have failed to demonstrate a critical role for NO in maintaining coronary blood flow during basal conditions. Thus, studies in anesthetized and awake dogs failed to show a decrease in basal coronary blood flow after NO synthase inhibition with intracoronary LNNA, L-NMMA, or L-NAME. However, there is evidence that NO can interact with other endogenous vasodilator systems. Thus, in isolated perfused guinea pig hearts, inhibition of NO production with L-NAME increased adenosine release from 23±5 pmol/min during control conditions to 41±7 pmol/min. In isolated rabbit hearts, L-NAME (30 μmol/L) caused a reduction in myocardial infarct size comparable to that produced by ischemic preconditioning; this protective effect was prevented by adenosine receptor blockade with 8-sulfophenyl theophylline, indicating that NO exerts its protective effect by increasing adenosine production. Although these studies demonstrate that NO synthase inhibitor can augment myocardial adenosine production, neither in anesthetized dogs nor in the present study did the addition of adenosine receptor blockade to L-NAME or LNNA cause a decrease in resting coronary flow. These findings demonstrate that in the normal blood-perfused heart in vivo neither adenosine nor NO alone or in combination is essential to maintain coronary flow during resting conditions.

Several investigators have examined the effect of K$_{ATP}$ channel blockade on basal coronary blood flow. Imamura et al. observed that intracoronary glibenclamide (50 μg/kg·min$^{-1}$ IC) caused a 40% decrease in basal coronary blood flow in open-chest dogs. Similarly, we observed that glibenclamide (50 μg/kg·min$^{-1}$ IC) caused a 20% to 30% decrease in coronary blood flow in resting awake dogs. After K$_{ATP}$ channel blockade had been established, the subsequent addition of adenosine receptor blockade did not further decrease coronary flow. However, in the presence of combined K$_{ATP}$ channel blockade and adenosine receptor blockade, inhibition of NO synthesis with LNNA caused a decrease in resting coronary flow, myocardial oxygen consumption, and systolic wall thickening. The decrease in...
myocardial oxygen consumption and systolic wall thickening was not the result of a direct negative inotropic effect, since restoration of coronary flow to the control level with sodium nitroprusside caused recovery of contractile performance. The findings support the conclusion that under resting conditions $K_{\text{ATP}}$ channels are the main mediators of metabolic dilation but that when these channels are blocked, NO contributes significantly to coronary dilation but cannot fully compensate for the loss of $K_{\text{ATP}}$ channel activity.

### Coronary Reactive Hyperemia

In isolated guinea pig hearts, open chest dogs, and awake dogs, NO synthase inhibition with LNNA, L-NMMA, or L-NAME decreased total reactive hyperemia flow principally by attenuating the late phase of the hyperemic response, with some investigators also reporting a decrease in the peak flow rate. The decrease in the late phase of reactive hyperemia likely occurred because shear-mediated NO-dependent vasodilation is delayed relative to metabolic small-vessel vasodilation. Thus, Hintze and Vatner observed that after release of a 15-second coronary occlusion, peak blood flow rates (metabolic resistance vessel dilation) occurred within 5 to 6 seconds, whereas flow-mediated vasodilation of the epicardial coronary artery required 45 to 60 seconds to occur. Similarly, Kuo et al reported that flow-mediated vasodilation required several minutes in perfused coronary microvessels. It is consequently not surprising that NO synthase blockade would attenuate the late phase of reactive hyperemia, when flow-mediated vasodilation would be expected to occur. However, after $K_{\text{ATP}}$ channel and adenosine receptor blockade in the present study, LNNA decreased both the peak blood flow rate and the duration of the response. This suggests that $K_{\text{ATP}}$ and adenosine blockade caused vasoconstriction, which increased endothelial shear and NO production, so that LNNA reduced the peak flow rate.

Combined blockade of $K_{\text{ATP}}$ channels, adenosine receptors, and NO synthase did not fully block reactive hyperemia. The residual vasodilation might be ascribed to incomplete blockade caused by the competitive inhibitors used in the present study. It is also possible that other vasodilators such as prostacyclin or endothelium–derived hyperpolarizing factor could be responsible for the residual vasodilation. Alternatively, metabolic alterations, such as decreased pH, increased $P_{\text{CO}_2}$ or direct sensing by arteriolar smooth muscle of the decreased oxygen tension during coronary occlusion, could contribute to the residual vasodilation. Finally, a part of the increase in coronary inflow can be accounted for by filling of coronary capacitance vessels, which were emptied during the occlusion. However, this could not account for the progressively greater increases in coronary inflow during reactive hyperemia that followed occlusions of longer duration.

### Coronary Blood Flow During Exercise

NO synthase blockade did not significantly alter coronary flow, possibly because of the relatively small number of animals studied, but it did decrease myocardial oxygen consumption during the heavier levels of exercise. Coronary venous oxygen tension tended to be lower after LNNA, suggesting that NO normally makes a modest contribution to coronary vasodilation during exercise. Nevertheless, LNNA combined with 8-PT did not alter coronary conductance during exercise, indicating that NO and adenosine are not essential for coronary vasodilation during exercise when $K_{\text{ATP}}$ channels are intact. In contrast, $K_{\text{ATP}}$ channel blockade with glibenclamide decreased coronary blood flow by 20% to 30% both at rest and during exercise but did not alter the slope of the relationship between coronary flow and myocardial oxygen demand. After $K_{\text{ATP}}$ channel blockade, the subsequent addition of adenosine blockade did not further decrease resting coronary blood flow but attenuated the increase in coronary flow during exercise by approximately half. This was accompanied by decreases in coronary venous oxygen tension and myocardial

### Table 4. Continued

<table>
<thead>
<tr>
<th>Corr $dP/dt_{\text{max}}$, mm Hg/s</th>
<th>Con</th>
<th>LNNA</th>
<th>L+8-PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>2730 ± 130</td>
<td>2540 ± 200</td>
<td>2620 ± 160</td>
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</tr>
<tr>
<td>3790 ± 210*</td>
<td>3490 ± 310*</td>
<td>3560 ± 250*</td>
<td></td>
</tr>
<tr>
<td>4070 ± 210*</td>
<td>3870 ± 240*</td>
<td>4040 ± 380*</td>
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<td>4600 ± 210*</td>
<td>4470 ± 190*</td>
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<td>5420 ± 300*</td>
<td>5318 ± 238*</td>
<td>5630 ± 410*</td>
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</tr>
</tbody>
</table>

### Table 5. Continued

<table>
<thead>
<tr>
<th>Myocardial Oxygen Consumption, mL/min</th>
<th>Con</th>
<th>LNNA</th>
<th>L+8-PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.9 ± 0.6</td>
<td>6.4 ± 0.7</td>
<td>6.8 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>9.3 ± 1.0*</td>
<td>11.2 ± 1.2</td>
<td>10.4 ± 0.8*</td>
<td></td>
</tr>
<tr>
<td>10.5 ± 1.1*</td>
<td>12.1 ± 1.1</td>
<td>13.1 ± 1.5*</td>
<td></td>
</tr>
<tr>
<td>11.5 ± 0.5*</td>
<td>14.8 ± 1.3†</td>
<td>13.7 ± 1.2†</td>
<td></td>
</tr>
<tr>
<td>12.7 ± 0.6*</td>
<td>16.2 ± 1.9†</td>
<td>16.3 ± 1.7†</td>
<td></td>
</tr>
</tbody>
</table>
contractile performance, which were most pronounced during the higher levels of exercise. These findings suggest that after K\textsuperscript{ATP} channel blockade, increasing levels of exercise are associated with progressive deterioration of the myocardial oxygen supply-demand balance, thereby resulting in progressive augmentation of adenosine production. Nevertheless, the addition of adenosine blockade after K\textsuperscript{ATP} channel blockade only partially attenuated the coronary vasodilation in response to exercise. However, after inhibition of K\textsuperscript{ATP} channels and adenosine receptors, the addition of NO synthase blockade essentially abolished coronary vasodilation during exercise. The residual tendency for coronary flow to increase in response to exercise was principally related to the increase in blood pressure without a significant increase in coronary conductance. Thus, NO-dependent mechanisms con-

### Table 6. Effects of Increasing Coronary Blood Flow by Intracoronary Infusion of Nitroprusside on Myocardial Oxygen Consumption and Regional Contractile Function During Resting Conditions

<table>
<thead>
<tr>
<th></th>
<th>Con Baseline</th>
<th>SNP Baseline</th>
<th>P+G Baseline</th>
<th>P+G+L Baseline</th>
<th>SNP Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>101±17</td>
<td>106±19</td>
<td>112±13</td>
<td>120±15</td>
<td>112±17</td>
</tr>
<tr>
<td>Mean aortic pressure, mm Hg</td>
<td>83±8</td>
<td>80±7</td>
<td>94±5</td>
<td>90±4</td>
<td>95±4</td>
</tr>
<tr>
<td>Coronary blood flow, mL/min</td>
<td>50±2</td>
<td>71±2*</td>
<td>37±4†</td>
<td>53±4*</td>
<td>29±4†</td>
</tr>
<tr>
<td>Myocardial oxygen consumption, mL/min</td>
<td>3.8±0.5</td>
<td>3.6±0.7</td>
<td>3.0±0.2†</td>
<td>3.8±0.6*</td>
<td>2.5±0.4†</td>
</tr>
<tr>
<td>LAD SWT, %</td>
<td>28±2</td>
<td>29±2</td>
<td>8±5†</td>
<td>26±2*</td>
<td>5±3†</td>
</tr>
<tr>
<td>LCX SWT, %</td>
<td>28±2</td>
<td>27±2</td>
<td>29±2</td>
<td>29±2</td>
<td>27±2</td>
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</table>

Con indicates control; P, 5 mg/kg IV 8-PT; G, 50 μg · kg\textsuperscript{-1} · min\textsuperscript{-1} IC glibenclamide; L, 1.5 mg/kg IC LNNA; SNP, 1.5 μg · kg\textsuperscript{-1} · min\textsuperscript{-1} sodium nitroprusside; LAD SWT, systolic wall thickening in the area perfused by the LAD; and LCX SWT, systolic wall thickening in the control area perfused by the left circumflex coronary artery. Values are mean±SEM (n=5).

*P<0.05 vs baseline; †P<0.05 vs corresponding Con measurements; ‡P<0.05 vs corresponding P+G measurements.
tribute to coronary vasodilation during exercise when other vasodilator systems have been inhibited.

The greater coronary vasorestriction produced by LNNA during exercise after K\textsuperscript{+}\textsubscript{ATP} channel blockade is analogous to the effect of NO synthase blockade on blood flow to myocardial regions perfused by a stenotic coronary artery. When a coronary stenosis caused myocardial hypoperfusion during exercise, intracoronary LNNA caused a further decrease in coronary flow with no change in perfusion pressure distal to the stenosis.\textsuperscript{3} This indicates that when a coronary stenosis results in myocardial ischemia during exercise, NO contributes to vasodilation of the distal vessels. Similarly, in the present study when the combination of glibenclamide and 8-PT caused myocardial hypoperfusion with contractile dysfunction, the addition of LNNA further reduced coronary blood flow. It is possible that blocking of K\textsuperscript{+}\textsubscript{ATP} channels and adenosine causes enhanced production of NO. NO production could be augmented by several mechanisms. First, the myocardial hypoperfusion produced by K\textsuperscript{+}\textsubscript{ATP} channel and adenosine blockade might directly increase NO production. In support of this, Pohl and Busse\textsuperscript{31} observed that hypoxia led to release of an endothelium-derived relaxing factor in feline mesenteric vessels. Similarly, myocardial hypoxia resulted in enhanced production of NO in isolated guinea pig hearts.\textsuperscript{21,22} It is thus possible that in the present study NO production was augmented by a direct effect of low tissue oxygen on the coronary endothelium.\textsuperscript{32} Another possible mechanism is that the vasorestriction produced by K\textsuperscript{+}\textsubscript{ATP} channel and adenosine blockade augmented NO release via an increase in shear stress.\textsuperscript{25} Finally, it is likely that NO normally contributes to coronary vasodilation during exercise but that compensation by other vasodilator systems acts to minimize the decrease in coronary blood flow produced by NO synthase blockade. Only when other vasodilator mechanisms are blocked does inhibition of NO synthase cause an absolute decrease of coronary flow. Future studies, including measurements of coronary arteriovenous NO production after K\textsuperscript{+}\textsubscript{ATP} channel blockade, will be needed to resolve these questions.

By comparing measurements of coronary conductance during combined blockade of K\textsuperscript{+}\textsubscript{ATP} channels, adenosine receptors, and NO synthase with measurements obtained when only K\textsuperscript{+}\textsubscript{ATP} channels and adenosine receptors were blocked, it is possible to estimate the influence of NO on coronary conductance. At rest, coronary conductance was 0.51±0.04 mL \cdot min\textsuperscript{-1} \cdot mm Hg\textsuperscript{-1} during control conditions; during combined K\textsuperscript{+}\textsubscript{ATP} channel and adenosine blockade, conductance decreased to 0.27±0.03 mL \cdot min\textsuperscript{-1} \cdot mm Hg\textsuperscript{-1} and fell further to 0.20±0.03 mL \cdot min\textsuperscript{-1} \cdot mm Hg\textsuperscript{-1} with the addition of LNNA, a decrease of 0.07 mL \cdot min\textsuperscript{-1} \cdot mm Hg\textsuperscript{-1} attributable to NO-dependent vasodilation. The decrease in coronary conductance following NO blockade was greater during exercise; during the highest level of exercise, conductance during control conditions was 0.93±0.08 mL \cdot min\textsuperscript{-1} \cdot mm Hg\textsuperscript{-1} and decreased to 0.43±0.05 mL \cdot min\textsuperscript{-1} \cdot mm Hg\textsuperscript{-1} in the presence of K\textsuperscript{+}\textsubscript{ATP} channel and adenosine blockade. Conductance fell to 0.24±0.04 mL \cdot min\textsuperscript{-1} \cdot mm Hg\textsuperscript{-1} with the addition of LNNA, a decrease of 0.19 mL \cdot min\textsuperscript{-1} \cdot mm Hg\textsuperscript{-1} attributable to NO-dependent vasodilation. The greater contribution of NO during exercise suggests that greater endothelial shear associated with the higher coronary flow rates during exercise augments NO production. Alternatively, sympathetic activation during exercise might enhance endothelial \(\alpha\textsubscript{2}\text{-adrenoceptor-mediated} \) NO production.\textsuperscript{33} LNNA could then aggravate hypoperfusion by leaving \(\alpha\textsubscript{2}\) - and \(\alpha\textsubscript{1}\text{-adrenergic} \) coronary vasoconstriction unopposed. This concept is in agreement with studies\textsuperscript{36} reporting that treadmill exercise caused epicardial artery dilation, which was converted to vasoconstriction after NO synthase inhibition or endothelial denudation, and is further supported by the finding that constriction of coronary microvessels in response to norepinephrine was enhanced after NO synthase inhibition.\textsuperscript{33}

**Limitations**

Interpretation of the role of K\textsuperscript{+}\textsubscript{ATP} channels in the regulation of coronary vascular resistance is predicated on the assumption that glibenclamide is not acting as a nonspecific constrictor of vascular smooth muscle. Any intervention that decreases coronary blood flow relative to myocardial oxygen demands (pharmacological vasoconstriction, arterial stenosis, or closing K\textsuperscript{+}\textsubscript{ATP} channels) likely results in recruitment of vasodilator mechanisms, which may not be mandatory for maintaining coronary vasodilation during normal arterial inflow. Khayyal et al\textsuperscript{34} demonstrated that when coronary flow was decreased to approximately half the basal level by intracoronary infusion of vasopressin, contractile force was decreased and lactate production occurred. Despite this evidence for ischemia, vasodilator reserve was not exhausted, as indicated by an increase in flow in response to adenosine. Endogenous vasodilator mechanisms also were not exhausted, since coronary occlusion resulted in some degree of reactive hyperemia. We observed analogous findings during K\textsuperscript{+}\textsubscript{ATP} channel blockade. Thus, glibenclamide decreased resting coronary blood flow but did not impair the ability of flow to increase during the metabolic stimulus produced by exercise. After glibenclamide, the ability to increase flow in response to exercise is mediated by adenosine and NO production in the heart. The results of the present study are unique because glibenclamide is not an agonist vasoconstrictor but acts to interrupt the common pathway for endogenous vasodilator mechanisms which require opening of K\textsuperscript{+}\textsubscript{ATP} channels to produce their effect (metabolic coronary vasodilation and autoregulation). Central to our hypothesis is the question of whether glibenclamide is a selective inhibitor of K\textsuperscript{+}\textsubscript{ATP} channels or whether it produces coronary vasoconstriction in a nonspecific manner. Glibenclamide produced a high degree of K\textsuperscript{+}\textsubscript{ATP} channel blockade without blunting the vasodilation produced by nitroprusside. In rat portal vein preparations, glibenclamide inhibited both K\textsuperscript{+}\textsubscript{ATP} channels and K\textsuperscript{+},\textsubscript{G\textsubscript{2+}} channels.\textsuperscript{35} However, in porcine coronary artery vascular smooth muscle cells\textsuperscript{36} and in isolated rat heart,\textsuperscript{37} there was no overlap in the pharmacological actions of glibenclamide and known blockers of K\textsuperscript{+},\textsubscript{G\textsubscript{2+}} channels, including large- and small-conductance K\textsuperscript{+},\textsubscript{G\textsubscript{2+}} channels (charybdotoxin) or blockers of the delayed rectifier K\textsuperscript{+} current (E-1403). Glibenclamide is generally regarded as the most potent and selective K\textsuperscript{+}\textsubscript{ATP} channel blocker available in coronary arterial vasculature.\textsuperscript{38} Nevertheless, we cannot exclude the alternate interpretation that closing K\textsuperscript{+}\textsubscript{ATP} channels with a pharmacological agent such as glibenclamide might constrict the vessels to a greater degree than simply withdrawing the
endogenous signals that cause vasodilation by opening these channels.

**Hierarchy of Coronary Vasodilator Systems**

Although coronary vasodilation produced by exercise was nearly abolished after combined blockade of K$_{\text{ATP}}$ channels, adenosine receptors, and NO synthase, blockade of any one of these vasodilator mechanisms alone failed to blunt the increase in coronary flow in response to exercise. However, LNNa tended to decrease coronary venous oxygen tension, suggesting that NO normally makes a modest contribution to coronary vasodilation, especially during low levels of exercise. After combined blockade of adenosine receptors and NO synthase, blood flow at rest and during exercise was not less than control flow. Although blockade of K$_{\text{ATP}}$ channels did not prevent the exercise-induced coronary vasodilation, it did result in lower coronary flow rates both at rest and during exercise, and this was associated with contractile dysfunction and increased myocardial release of adenosine, suggestive of ischemia. These findings are best explained by the concept that opening of K$_{\text{ATP}}$ channels is the principal mechanism of metabolic coronary vasodilation but that when K$_{\text{ATP}}$ channels are blocked and ischemia ensues, both adenosine and NO act to increase coronary blood flow during exercise. When K$_{\text{ATP}}$ channels and adenosine receptors were blocked, NO produced approximately one quarter of the coronary vasodilation that usually occurred in response to exercise when all vasodilator mechanisms were intact.

**Intrinsic Coronary Vascular Tone**

When all three vasodilator mechanisms were blocked, coronary blood flow both at rest and during exercise was reduced below the level observed at rest during control conditions. This finding suggests that the intrinsic state of the coronary resistance vessels is one of marked vasoconstriction, with messengers generated by the myocardial myocytes and endothelium acting to cause vasoconstriction. α-Adrenergic activation contributes to coronary vasoconstriction during exercise, but this mechanism is probably of negligible importance under resting conditions. Consequently, the high level of vasomotor tone is likely an intrinsic characteristic of the coronary resistance vessels. Skeletal muscle resistance vessels also maintain a high level of vasoconstrictor tone during basal conditions that has been attributed to myogenic mechanisms. It is unclear whether similar myogenic mechanisms also exist in the coronary resistance vessels.

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ATP-Sensitive K⁺ Channels, Adenosine, and Nitric Oxide–Mediated Mechanisms Account for Coronary Vasodilation During Exercise
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