Role of Adenosine in Coronary Vasodilation During Exercise

Robert J. Bache, Xue-Zheng Dai, Jeffrey S. Schwartz, and David C. Homans

This study examined the hypothesis that increases in myocardial blood flow during exercise are mediated by adenosine-induced coronary vasodilation. Active hyperemia associated with graded treadmill exercise and coronary reactive hyperemia were examined in chronically instrumented awake dogs during control conditions, after intracoronary infusion of adenosine deaminase (5 units/kg/min for 10 minutes), and after adenosine receptor blockade with 8-phenyltheophylline. Both adenosine deaminase and 8-phenyltheophylline caused a rightward shift of the dose-response curve to intracoronary adenosine; 8-phenyltheophylline was significantly more potent than adenosine deaminase. Adenosine deaminase caused a 33 ± 7 to 39 ± 3% decrease in reactive hyperemia blood flow following coronary occlusions of 5–20 seconds duration, respectively, while 8-phenyltheophylline produced a 40 ± 6 to 62 ± 8% decrease in reactive hyperemia. Increasing myocardial oxygen consumption during treadmill exercise was associated with progressive increases of coronary blood flow. Neither adenosine deaminase nor 8-phenyltheophylline attenuated the increase in coronary blood flow or the decrease of coronary vascular resistance during exercise. Neither agent altered the relation between myocardial oxygen consumption and coronary blood flow. Thus, although both adenosine deaminase and 8-phenyltheophylline antagonized coronary vasodilation in response to exogenous adenosine and blunted coronary reactive hyperemia, neither agent impaired coronary vasodilation associated with increased myocardial oxygen requirements produced by exercise. These findings fail to support a substantial role for adenosine in mediating coronary vasodilation during exercise. (Circulation Research 1988;62:846–853)

The adenosine hypothesis proposes that adenosine produced from intracellular adenine nucleotide stores is continuously released from myocardial cells into the interstitial fluid and that during conditions of increased myocardial oxygen demands or reduced arterial oxygen supply release of adenosine is increased. Adenosine is a potent coronary vasodilator that interacts with a specific receptor on the coronary myocyte to result in relaxation of the vascular smooth muscle. In this way, adenosine could function as a messenger to couple oxygen requirements of the myocardium to vasomotor tone of the coronary resistance vessels and, therefore, delivery of arterial blood to the myocardium.

Studies of the importance of adenosine in regulating coronary blood flow have used either intracoronary administration of adenosine deaminase catalytic subunits to degrade adenosine released into the cardiac interstitial space or adenosine receptor blockers such as theophylline. Several recent studies have failed to demonstrate a significant role for adenosine in maintaining blood flow or influencing coronary autoregulation during conditions of basal myocardial oxygen requirements. Myocardial adenosine content has been shown to increase during exercise, suggesting that increased adenosine production could contribute to the increased coronary blood flow during exercise. Consequently, the present study was carried out to assess the contribution of adenosine to the coronary vasodilation and active hyperemia that occurs during exercise. Studies were performed in chronically instrumented dogs trained to run on a motor-driven treadmill. An initial study examined the effect of intracoronary adenosine deaminase and compared this response with intravenous administration of the adenosine receptor antagonist 8-phenyltheophylline. Because the use of intracoronary adenosine deaminase did not allow coronary venous blood sampling in the area of myocardium corresponding to the region of adenosine inactivation, a second study was performed in which systemic administration of 8-phenyltheophylline was used to provide uniform myocardial adenosine blockade and, therefore, allow examination of the effects of adenosine blockade on coronary venous oxygen tension.

Materials and Methods

Studies were performed in 15 adult mongrel dogs (24–28 kg) trained to run on a motor-driven treadmill. The animals were anesthetized with sodium pentobarbital (25–30 mg/kg i.v.), intubated, and ventilated with a respirator. A thoracotomy was performed in the fifth left intercostal space, and the heart suspended in a pericardial cradle. A PVC catheter (3.0 mm o.d.) filled with heparin-saline solution (200 units/ml) was introduced into the left internal thoracic artery and advanced into the ascending aorta. Similar catheters were introduced into the left atrial cavity via the atrial appendage and the left ventricular cavity through a stab wound in
the apical dimple; catheters were secured with purse-string sutures. The proximal 2.5 cm of the left circumflex coronary artery was dissected free, and a Statham SP-type electromagnetic flowmeter probe was positioned around the vessel. An inflatable cuff-type hydraulic occluder was placed around the artery distal to the flowmeter probe but proximal to any arterial branches. For dogs in Group 1, a Tygon microbore catheter (0.04 inches o.d.) was introduced into the left circumflex coronary artery using the technique of Gould and associates. For dogs in Group 2 in which coronary sinus blood sampling was required, a PVC catheter (3.0 mm o.d.) was introduced into the right coronary sinus ostium, advanced until it could be palpated within the coronary sinus on the lateral surface of the heart, and secured in place with a purse-string suture. The catheters, occluder tubing, and flowmeter leads were tunneled dorsally to exit through the skin at the base of the neck. The pericardium was loosely closed, the thoracotomy incision repaired, and the dogs allowed to recover. The catheters, flowmeter leads, and occluder tubing were protected with a nylon vest the dogs had been trained to wear (Alice King Chatham, Los Angeles, California). Catheters were flushed daily with heparinized saline. Exercise training on the treadmill was reinstated 7–10 days after surgery. Studies were performed 12–15 days after surgery. Aortic, left atrial, and left ventricular pressures were measured with Statham P23ID pressure transducers attached to the nylon vest at midchest level. Coronary blood flow was measured with a Statham SP2202 electromagnetic flowmeter. The zero flow baseline for the electromagnetic flowmeter was determined with 2–3-second occlusions of the left circumflex coronary artery. Data were recorded on a Hewlett-Packard Model 8800 eight-channel direct writing oscillograph.

Group 1

Group 1 consisted of seven dogs with indwelling catheters in the left circumflex coronary artery to allow intra-arterial drug infusion. After all recording instruments were connected, the animals were allowed to rest quietly on the treadmill for 30 minutes. Hemodynamic variables were continuously monitored during this interval to ensure that steady-state resting conditions had been achieved. After obtaining resting hemodynamic measurements, a 3-minute period of warmup exercise was begun at a treadmill speed of 3.2 km/hr with 0% grade. Immediately thereafter, a five-stage treadmill exercise protocol shown in Table 3 was begun. Each exercise stage was 3 minutes in duration; all pressures and coronary blood flow were measured during the third minute of each exercise stage. At the conclusion of exercise, a 2–3-second coronary artery occlusion was performed to ensure that the coronary flowmeter baseline had remained stable throughout the period of exercise. Data were rejected if a >5% shift in the zero flow measurement occurred.

At the conclusion of exercise, the dog was allowed to rest quietly on the treadmill until heart rate, pressure, and coronary blood flow had returned to the preexercise control level; this generally occurred within 5–10 minutes after the end of exercise. Then, the reactive hyperemic responses to coronary occlusions 5, 10, and 20 seconds in duration were observed in duplicate. A 3-minute interval was allowed between occlusions. Immediately thereafter, the response of coronary blood flow to intracoronary bolus administration of adenosine in doses of 5, 10, and 20 μg was observed. Adenosine was dissolved in normal saline, and multiple dilutions were prepared so that the volume injected did not exceed 0.25 ml. The change in coronary blood flow that occurred in response to administration of a similar volume of normal saline was subtracted from the increase in flow caused by adenosine.

After allowing a 1-hour rest period, adenosine deaminase was infused into the left circumflex coronary artery. Type I adenosine deaminase from calf intestine (Sigma Chemical, St. Louis, Missouri) was dissolved in Krebs-Henseleit buffer, pH 7.4, and infused at a dose of 5 units/kg body wt/min (infusion rate, 1.23 ml/min) for 10 minutes. At the conclusion of the infusion, the above protocol was repeated beginning with the five-stage graded exercise protocol, followed by reactive hyperemia responses to 5-, 10-, and 20-second occlusions, and then adenosine dose-response curves. All measurements were completed within 1 hour after completion of adenosine deaminase infusion.

After completing this study, dose-response curves to intracoronary bolus doses of adenosine were observed at hourly intervals. When the dose-response curve to adenosine had returned to control, adenosine receptor blockade was produced by intravenous administration of 8-phenyltheophylline (5 mg/kg). Ten minutes after administration of 8-phenyltheophylline, the above protocol was repeated, beginning with the graded treadmill exercise protocol, followed by reactive hyperemia following 5-, 10-, and 20-second coronary occlusions, and finally adenosine dose-response curves.

Group 2

The eight dogs in Group 2 were instrumented with coronary sinus catheters to allow measurement of coronary sinus oxygen tension and determination of myocardial oxygen consumption. Because it was not possible to obtain coronary venous drainage corresponding to the region of the left circumflex coronary artery without admixture with blood from other myocardial areas, the adenosine receptor antagonist was administered systemically to produce uniform effects on the myocardium. When the animals had achieved steady-state hemodynamic conditions while standing quietly on the treadmill, aortic and coronary sinus blood samples (1.0 ml each) were withdrawn anaerobically for measurement of hemoglobin, $P_{O_2}$, $P_{CO_2}$, and pH. Immediately thereafter, the five-stage exercise protocol described for the Group 1 animals was performed. Ten minutes after completion of exercise, the reactive hyperemic responses to 10- and 20-second coronary artery occlusions were observed in duplicate.
A 1–2-hour rest period was allowed after completion of exercise. The adenosine receptor antagonist 8-phenyltheophylline was then administered in a dosage of 5 mg/kg i.v. over 2 minutes. Hemodynamic measurements were monitored continuously. Ten minutes after administration of 8-phenyltheophylline, the five-stage exercise protocol was repeated, and aortic and coronary sinus blood specimens were withdrawn during the third minute of each exercise stage. Ten minutes after completion of exercise, reactive hyperemic responses were repeated.

Blood specimens were maintained in iced syringes until the conclusion of each exercise protocol. Measurements of P O2, P CO2, and pH were then immediately performed with an Instrumentation Laboratory Model 113 blood gas analyzer (Lexington, Massachusetts) previously calibrated with known gas mixtures. Hemoglobin content was determined with the cyanmethemoglobin method. Hemoglobin saturation was estimated from the blood Po2, pH, and temperature using the oxygen dissociation curve for dog blood. Blood oxygen content was calculated as Hb x 1.34 x % O2 saturation + (0.0031 x Po2). Oxygen consumption in the region of myocardium perfused by the left circumflex coronary artery was computed as the product of flow measured with the electromagnetic flowmeter and the difference in oxygen content between aortic and coronary sinus blood.

Data Analysis

Heart rate, pressure, and mean coronary blood flow were measured from the strip chart recordings. Total blood flow during reactive hyperemia was determined by electrical integration of the electromagnetic flowmeter tracing. Blood flow debt, reactive hyperemia flow, and blood flow debt repayment were calculated as described by Olsson and Gregg: blood flow debt (ml) = control flow rate (ml/sec) x duration of occlusion (sec); reactive hyperemia flow = total flow during reactive hyperemia (ml) – [control flow rate (ml/sec) x duration of reactive hyperemia (sec)]; blood flow debt repayment (%) = [reactive hyperemia flow (ml)/blood flow debt (ml)] x 100.

Hemodynamic data, blood gas measurements, and myocardial oxygen consumption data obtained during exercise were compared using two-way analysis of variance testing for the effects of exercise and drug treatment. A value of p < 0.05 was required for statistical significance. When significant differences were found, multiple contrasts were performed; p values were adjusted using the Bonferroni method, which corrects for performing multiple comparisons on correlated data. Comparisons of the measurements obtained during reactive hyperemia were performed using Student's t test for paired data; p values were corrected using the Bonferroni method.

Results

Group 1

During quiet resting conditions, intracoronary infusion of adenosine deaminase caused no significant change in heart rate, arterial pressure, or coronary blood flow. Intravenous administration of 8-phenyltheophylline caused a transient increase in mean arterial pressure from 94 ± 4 to 98 ± 4 mm Hg (p < 0.05); arterial pressure returned to the control level in 5.2 ± 1.4 minutes after administration of 8-phenyltheophylline. Heart rate and coronary blood flow did not change significantly in response to 8-phenyltheophylline.

The response to intracoronary adenosine is shown in Table 1 and Figure 1. During control conditions, adenosine administration resulted in prompt dose-related increases of coronary blood flow. Adenosine deaminase caused 86 ± 13% inhibition of the increase in total flow volume produced by adenosine (5 μg) and 73 ± 11% inhibition of the response to adenosine (20 μg) (Figure 1). 8-Phenyltheophylline produced 95% or greater inhibition of coronary vasodilation in response to exogenous adenosine at all doses tested and was significantly more potent than adenosine deaminase at doses of 10 and 20 μg of adenosine (p < 0.01).

Reactive hyperemia data are shown in Table 2. During control conditions, reactive hyperemia blood flow debt repayments were 431 ± 64% following 5-second occlusions and 502 ± 77% following 20-second occlusions. Adenosine deaminase resulted in significant reductions of percent debt repayment following occlusions of 10 and 20 seconds duration. 8-Phenyltheophylline depressed reactive hyperemia to a greater extent than adenosine deaminase, causing least inhibition of coronary blood flow.

### Table 1. Response of Left Circumflex Coronary Artery Blood Flow to Intracoronary Adenosine in Seven Dogs in Group 1

<table>
<thead>
<tr>
<th></th>
<th>Adenosine</th>
<th></th>
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<tr>
<td></td>
<td>5 μg</td>
<td>10 μg</td>
<td>20 μg</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>ADA</td>
<td>8-PT</td>
<td>CON</td>
<td>ADA</td>
<td>8-PT</td>
<td>CON</td>
<td>ADA</td>
<td>8-PT</td>
<td>CON</td>
</tr>
<tr>
<td>Control flow (ml/min)</td>
<td>52 ± 9</td>
<td>55 ± 9</td>
<td>56 ± 7</td>
<td>53 ± 9</td>
<td>59 ± 13</td>
<td>56 ± 12</td>
<td>53 ± 8</td>
<td>60 ± 13</td>
<td>56 ± 13</td>
<td></td>
</tr>
<tr>
<td>Peak flow (ml/min)</td>
<td>147 ± 27</td>
<td>87 ± 17*</td>
<td>58 ± 12*</td>
<td>172 ± 29</td>
<td>104 ± 23*</td>
<td>64 ± 12†</td>
<td>196 ± 25</td>
<td>122 ± 30*</td>
<td>74 ± 15†</td>
<td></td>
</tr>
<tr>
<td>Duration (seconds)</td>
<td>26 ± 2</td>
<td>7 ± 3*</td>
<td>1 ± 1*</td>
<td>28 ± 2</td>
<td>12 ± 5*</td>
<td>5 ± 2*</td>
<td>43 ± 4</td>
<td>17 ± 3*</td>
<td>9 ± 2†</td>
<td></td>
</tr>
</tbody>
</table>

Control flow rates prior to adenosine administration as well as the peak blood flow and duration of the hyperemia produced by adenosine are shown.

Values are mean ± SEM.

* p < 0.05 compared with corresponding control measurement; † p < 0.05 compared with corresponding measurement after adenosine deaminase.
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40-
20-
0-
40-
20-
0-
40-
20-
0-
40-
20-
0-

Figure 1. Dose-response curves to intracoronary bolus doses of adenosine in seven dogs in Group 1. Absolute increases in left circumflex coronary artery blood flow volume in response to adenosine are compared during control conditions, after intracoronary infusion of adenosine deaminase (5 units/kg/min for 10 minutes), and after adenosine receptor blockade with 8-phenyltheophylline (5 mg/kg i.v.).

TABLE 2. Reactive Hyperemia Following Occlusions of Left Circumflex Coronary Artery of 5-, 10-, and 15-Seconds Duration in Seven Dogs in Group 1

<table>
<thead>
<tr>
<th>Occlusion</th>
<th>5-Seconds</th>
<th>10-Seconds</th>
<th>20-Seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>ADA</td>
<td>8-PT</td>
</tr>
<tr>
<td>Control flow (ml/min)</td>
<td>56 ± 9</td>
<td>61 ± 5</td>
<td>60 ± 9</td>
</tr>
<tr>
<td>Flow debt (ml)</td>
<td>4.7 ± 0.8</td>
<td>5.0 ± 0.8</td>
<td>5.2 ± 0.8</td>
</tr>
<tr>
<td>Reactive hyperemia duration (seconds)</td>
<td>28 ± 5</td>
<td>23 ± 3</td>
<td>15 ± 7</td>
</tr>
<tr>
<td>Peak flow (ml/min)</td>
<td>168 ± 26</td>
<td>174 ± 34</td>
<td>152 ± 29</td>
</tr>
</tbody>
</table>
| Repayment (%) | 431 ± 64  | 351 ± 83  | 242 ± 30* | 424 ± 49  | 305 ± 61* | 202 ± 40* | 502 ± 77  | 308 ± 25* | 188 ± 39* | 5

Values are mean ± SEM.

CON, control; ADA, adenosine deaminase; 8-PT, 8-phenyltheophylline.
*p<0.05 compared with corresponding control measurement; †p<0.05 compared with corresponding measurement after adenosine deaminase.
TABLE 4. Hemodynamic Data Including Myocardial Oxygen Consumption and Coronary Sinus Oxygen Tension From Eight Dogs in Group 2 at Rest and During Graded Treadmill Exercise

<table>
<thead>
<tr>
<th>Exercise (speed/grade)</th>
<th>Heart rate (beats/min)</th>
<th>Mean aortic pressure (mm Hg)</th>
<th>Coronary blood flow (ml/min)</th>
<th>Myocardial O₂ consumption (ml/min)</th>
<th>Coronary sinus O₂ tension (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>ADA</td>
<td>8-PT</td>
<td>CON</td>
<td>ADA</td>
</tr>
<tr>
<td>Rest</td>
<td>127 ± 8</td>
<td>118 ± 6</td>
<td>123 ± 7</td>
<td>103 ± 3</td>
<td>95 ± 5</td>
</tr>
<tr>
<td>4.8 km/hr, 0%</td>
<td>171 ± 6</td>
<td>169 ± 6</td>
<td>165 ± 5</td>
<td>105 ± 3</td>
<td>102 ± 4</td>
</tr>
<tr>
<td>6.4 km/hr, 5%</td>
<td>196 ± 8</td>
<td>190 ± 6</td>
<td>191 ± 6</td>
<td>109 ± 3</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>6.4 km/hr, 10%</td>
<td>208 ± 7</td>
<td>204 ± 8</td>
<td>207 ± 7</td>
<td>114 ± 3</td>
<td>109 ± 4</td>
</tr>
<tr>
<td>6.4 km/hr, 15%</td>
<td>220 ± 9</td>
<td>219 ± 10</td>
<td>223 ± 9</td>
<td>118 ± 3</td>
<td>112 ± 4</td>
</tr>
<tr>
<td>6.4 km/hr, 20%</td>
<td>241 ± 8</td>
<td>240 ± 7</td>
<td>239 ± 7</td>
<td>129 ± 6</td>
<td>118 ± 6</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

CON, control; ADA, adenosine deaminase; 8-PT, 8-phenyltheophylline.

(p<0.01). There was a direct linear relation between coronary blood flow and myocardial oxygen consumption during exercise. During control conditions, coronary sinus oxygen tension decreased significantly during exercise. Adenosine receptor blockade with 8-phenyltheophylline did not alter myocardial oxygen consumption, coronary blood flow, or coronary sinus oxygen tension either at rest or during exercise and did not alter the relation between myocardial oxygen consumption and coronary blood flow.

Because animals in Group 2 did not have indwelling coronary artery catheters, adenosine dose-response curves were not performed. However, coronary reactive hyperemic responses following 10- and 20-second occlusions of the left circumflex coronary artery were obtained. During control conditions, reactive hyperemia following 10-second coronary occlusions resulted in 501 ± 74% debt repayment; this was reduced to 264 ± 47% after 8-phenyltheophylline (p<0.02). Blood flow debt repayment following 20-second coronary occlusions was 407 ± 67% during control conditions and was decreased to 290 ± 60% after 8-phenyltheophylline (p<0.02). These responses were not different from those observed in animals in Group 1 following 8-phenyltheophylline.

Discussion
In the present study, neither enhanced adenosine degradaton produced by intracoronary administration of adenosine deaminase nor adenosine receptor blockade with 8-phenyltheophylline altered myocardial blood flow at rest or blunted the increase in coronary flow during submaximal treadmill exercise. These findings fail to support an important role for adenosine in mediating coronary vasodilation in response to physiological increases of myocardial oxygen demands that occur during exercise.

Methylxanthines act as competitive inhibitors of adenosine. However, these compounds also possess phosphodiesterase-blocking properties. Consequently, the degree of adenosine antagonism that can be achieved with these agents in physiological systems is limited by the increases in heart rate, cardiac contractility, and myocardial oxygen consumption, which they produce at high dosages. Previous studies of the effects of adenosine receptor blockade on the coronary circulation have used theophylline or aminophylline. Burger et al. using isolated perfused guinea pig hearts, found that competitive inhibition of adenosine by theophylline was incomplete so that 10^-4 M adenosine, a concentration that produced 85% of maximal coronary vasodilation, was only 20% inhibited by 5.5 x 10^-3 M theophylline; higher concentrations of theophylline caused increased cardiac contractility. Merrill et al. obtained similar results. In the present study, 8-phenyltheophylline was used to take advantage of the greater adenosine-blocking activity of this agent. In a study of xanthine analogues, Smelie et al. obtained similar results.

TABLE 4. Hemodynamic Data Including Myocardial Oxygen Consumption and Coronary Sinus Oxygen Tension From Eight Dogs in Group 2 at Rest and During Graded Treadmill Exercise

<table>
<thead>
<tr>
<th>Exercise (speed/grade)</th>
<th>Heart rate (beats/min)</th>
<th>Mean aortic pressure (mm Hg)</th>
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<th>Coronary sinus O₂ tension (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>8-PT</td>
<td>CON</td>
<td>8-PT</td>
<td>CON</td>
</tr>
<tr>
<td>Rest</td>
<td>113 ± 7</td>
<td>97 ± 4</td>
<td>98 ± 5</td>
<td>29 ± 4</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>4.8 km/hr, 0%</td>
<td>175 ± 7</td>
<td>105 ± 5</td>
<td>109 ± 5</td>
<td>45 ± 6</td>
<td>50 ± 8</td>
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<tr>
<td>6.4 km/hr, 5%</td>
<td>205 ± 8</td>
<td>114 ± 4</td>
<td>114 ± 4</td>
<td>59 ± 8</td>
<td>63 ± 9</td>
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<tr>
<td>6.4 km/hr, 10%</td>
<td>222 ± 8</td>
<td>116 ± 4</td>
<td>118 ± 3</td>
<td>69 ± 7</td>
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<td>6.4 km/hr, 15%</td>
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<tr>
<td>6.4 km/hr, 20%</td>
<td>264 ± 7</td>
<td>128 ± 5</td>
<td>130 ± 2</td>
<td>91 ± 9</td>
<td>91 ± 10</td>
</tr>
</tbody>
</table>

Measurements were obtained at rest and after adenosine receptor blockade with 8-phenyltheophylline (5 mg/kg i.v.).

Values are mean ± SEM.

CON, control; 8-PT, 8-phenyltheophylline.
found that 8-phenylethophylline was the most potent available adenosine antagonist while having relatively low activity as an inhibitor of calcium-dependent phosphodiesterase. Using isolated vascular ring segments of rabbit basilar artery to evaluate adenosine receptor antagonism, Griffiths et al.\(^{18}\) found a potency ratio calculated from PA\(_2\) values of more than 100:1 when 8-phenylethophylline was compared with aminophylline. The dose of adenosine receptor antagonist is of critical importance; it is necessary to achieve a high degree of adenosine receptor blockade without causing sufficient phosphodiesterase inhibition to produce unwanted cardiac effects. These conditions were met in the present study; 8-phenylethophylline resulted in 95 ± 1.2% inhibition of the excess flow volume in response to 20 μg adenosine intracoronary (a dose of adenosine that resulted in an increase in flow similar to that during reactive hyperemia following a 10-second coronary occlusion) without causing significant alterations of heart rate, arterial pressure, or myocardial oxygen consumption.

In the present study, the adenosine receptor antagonist was administered systemically to allow evaluation of the effect of adenosine blockade on the relation between myocardial oxygen consumption and coronary blood flow during exercise. Intracoronary administration of the adenosine antagonist would affect only the resistance vessels within the perfusion bed of that coronary artery. Coronary sinus sampling would then represent a mixture of venous blood from areas of myocardium with blocked and unblocked adenosine receptors and could not be used to examine the effects of adenosine blockade on myocardial oxygen consumption or coronary venous oxygen tension. To avoid this problem, 8-phenylethophylline was administered systemically. To ensure that systemic administration of 8-phenylethophylline resulted in a high degree of adenosine antagonism, in Group 1 dogs the effects of intracoronary adenosine deaminase were compared with systemically administered 8-phenylethophylline. Adenosine deaminase was administered by the intracoronary route at a dose previously shown to achieve cardiac lymph concentrations of 3 units/ml.\(^{14}\) Myocardial interstitial concentrations of adenosine deaminase of this magnitude would degrade even greatly increased quantities of adenosine.\(^{15,16}\) Although a high degree of adenosine antagonism was achieved, Rubio\(^{19}\) has pointed out that it may not be possible to reduce interstitial adenosine concentrations to subthreshold levels and thereby completely interrupt the vascular response to adenosine. Intravenous 8-phenylethophylline produced greater antagonism of coronary vasodilation in response to exogenous adenosine as well as a tendency toward greater blunting of coronary reactive hyperemia (which was statistically significant following 20-second coronary occlusions). Although the pharmacokinetics of this agent are not well described, these data indicate that 8-phenylethophylline did achieve adequate concentrations to produce a high degree of antagonism to exogenous and endogenous adenosine.

Neither adenosine deaminase nor 8-phenyletholphyl- line significantly altered coronary vascular resistance or myocardial oxygen extraction during quiet resting conditions. This is in agreement with previous studies that have failed to demonstrate an important role for adenosine in regulating coronary blood flow during basal conditions. Thus, Saito et al.\(^{3}\) and Kroll and Fieg\(^{6}\) found that adenosine deaminase caused no change in basal myocardial blood flow in open-chest dogs, while Gewitz et al.\(^{7}\) reported that intracoronary adenosine deaminase produced only a slight increase in resting coronary blood flow in closed-chest pigs. Similarly, Hanley et al.\(^{7}\) found that coronary pressure-flow relations were unchanged after treatment with adenosine deaminase, indicating that inactivation of adenosine did not cause loss of coronary autoregulation in open-chest dogs.

Despite general agreement that adenosine does not contribute to maintenance of coronary vasomotor tone during basal conditions, circumstantial evidence has suggested that adenosine may contribute to coronary vasodilation in response to increased myocardial metabolic needs. Watkinson et al.\(^{19}\) and Ely et al.\(^{20}\) using dogs with chronically implanted pericardial catheters, found increased pericardial infusate adenosine concentrations as evidence of increased myocardial adenosine production during treadmill exercise. McKenzie et al.\(^{10}\) obtained myocardial biopsies during treadmill exercise in chronically instrumented dogs. Exercise caused increases in myocardial adenosine content that were negatively correlated with coronary vascular resistance and were associated with increased adenosine concentrations in coronary sinus blood. These increases of myocardial and coronary venous adenosine appeared to be normal physiological responses to exercise because persistently low myocardial lactate concentrations indicated no evidence for myocardial ischemia. It should be emphasized that myocardial adenosine concentration may not reflect extracellular adenosine concentrations to which the coronary arterioles are exposed because a substantial fraction of myocardial adenosine resides in the intracellular compartment.\(^{21}\) However, increased adenosine concentration in coronary sinus blood and pericardial fluid indicate that interstitial adenosine concentration is also increased during exercise. The present study was undertaken to determine whether enhanced degrada-
tion of interstitial adenosine or antagonism of the effects of adenosine on the coronary resistance vessels would impair the increase in coronary blood flow that occurs during exercise. No such alteration was found. Neither adenosine deaminase nor 8-phenyltheophylline blunted the increase in coronary blood flow or the decrease in coronary vascular resistance that occurred during exercise. Adenosine receptor blockade with 8-phenyltheophylline did not alter the relation between myocardial oxygen consumption and coronary blood flow and did not cause decreased coronary venous oxygen tension during exercise, which would accompany even slight impairment of the increase in coronary blood flow during exercise. These findings fail to support a substantial role for adenosine in mediating coronary dilation during exercise.

Several previous studies have examined the importance of adenosine in mediating coronary vasodilation in response to increases of myocardial oxygen demands produced by interventions other than exercise. In open-chest dogs, Randall and Jones reported that adenosine receptor blockade with aminophylline attenuated the increase in coronary blood flow and exaggerated the increase in oxygen extraction that occurred during cardiac pacing but did not alter the increase in coronary flow when myocardial oxygen consumption was increased by intracoronary infusion of isoproterenol. Similarly, McKenzie et al found that theophylline did not attenuate the increase in coronary blood flow produced by isoproterenol in open-chest dogs; however, myocardial adenosine content was nearly doubled after theophylline administration. The investigators suggested that adenosine receptor blockade caused increased myocardial adenosine production, which may have compensated (at least in part) for the competitive inhibition produced by theophylline. Although Jones et al also found that the increase in coronary blood flow produced by isoproterenol was not attenuated by theophylline, these investigators found no increase in myocardial adenosine concentration after theophylline administration. The reason for the differing results between these two studies is unclear. However, because doses of theophylline that do not cause increases in heart rate, myocardial contractility, or myocardial oxygen consumption do not produce a high degree of adenosine receptor blockade, moderate increases in interstitial adenosine concentration might overcome the competitive inhibition produced by theophylline. In the present study, adenosine receptor blockade was produced with 8-phenyltheophylline, which is approximately 100 times more potent than theophylline in vascular tissue. It is unlikely that sufficient increases of myocardial adenosine could have been achieved to overcome the high degree of blockade produced by this agent.

Previous studies in which coronary reactive hyperemia was examined after adenosine receptor blockade with aminophylline have yielded conflicting results. Thus, using anesthetized open-chest dogs, several investigators reported 19-42% reductions of reactive hyperemia blood flow following coronary occlusions of 5-30 seconds duration. While others found that intravenous or intracoronary aminophylline did not significantly decrease coronary reactive hyperemia. In some of these studies, failure to observe a decrease in reactive hyperemia may have been the result of inadequate doses of theophylline. In addition, the use of aminophylline rather than theophylline in some of the studies could have introduced variability. Aminophylline is a 2:2 complex of ethylene diamine with theophylline; it is possible that ethylene diamine, which is a strong organic base, could by itself alter coronary reactivity. In studies performed in open-chest animals, the variable effects of general anesthesia and acute surgical trauma on the coronary circulation could have affected the results. In a previous study in chronically instrumented awake dogs, Radford et al found that aminophylline (5 mg/kg i.v.) caused a 19% decrease in reactive hyperemia blood flow following 10-second occlusions of the left circumflex coronary artery. As in the present study, the early portion of the reactive hyperemic response was not substantially altered by aminophylline, while the duration of reactive hyperemia was significantly decreased. Thus, the results obtained by Radford et al were qualitatively similar to the present study, although the absolute decrease in reactive hyperemia was less. The greater reduction of reactive hyperemia in the present study was likely related to the greater potency of 8-phenyltheophylline as an adenosine antagonist. In a study in open-chest dogs, Saito et al found that intracoronary infusion of adenosine deaminase (4.5 units/kg/min) reduced reactive hyperemia volume flow by 30-39%. This is similar to the 33-39% decrease in reactive hyperemic blood flow produced by adenosine deaminase in the present study.

Both adenosine deaminase and 8-phenyltheophylline caused a change in the pattern of reactive hyperemia with little effect on the initial vasodilation and peak flow response but with earlier recovery of vasoconstrictor tone and reduction in the duration of the response. This suggests that the initial part of the response may be mediated by factors other than adenosine, while adenosine plays a role in maintaining coronary vasodilation during the later part of the reactive hyperemia. In the present study, the decrease in reactive hyperemia blood flow was significantly more marked after adenosine receptor blockade with 8-phenyltheophylline than with adenosine deaminase. This is in agreement with the finding that 8-phenyltheophylline caused significantly greater blockade of the response to exogenous adenosine than did adenosine deaminase. Nevertheless, the reductions of peak and total blood flow during reactive hyperemia were much less than the reductions in response to exogenous adenosine that produced similar degrees of coronary vasodilation, suggesting that vasodilator mechanisms other than adenosine contribute to the reactive hyperemia that follows brief coronary occlusions.

In summary, both adenosine deaminase and 8-phenyltheophylline antagonized coronary vasodilation pro-
duced by exogenous adenosine and attenuated the reactive hyperemic response that followed 10- and 20-second periods of coronary artery occlusion. However, neither agent altered the increase in coronary blood flow or the decrease in coronary venous oxygen tension that occurred during treadmill exercise. The finding that doses of adenosine deaminase and 8-phenyltheophylline that were sufficient to attenuate coronary reactive hyperemia had no effect on exercise-induced hyperemia indicates that adenosine does not have an important role in mediating the coronary vasodilation that occurs during exercise.

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References


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