Endogenous Adenosine Mediates Coronary Vasodilation during Exercise after K^+_{ATP} Channel Blockade

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Abstract

The mechanism of coronary vasodilation produced by exercise is not understood completely. Recently, we reported that blockade of vascular smooth muscle K^+_{ATP} channels decreased coronary blood flow at rest, but did not attenuate the increments in coronary flow produced by exercise. Adenosine is not mandatory for maintaining basal coronary flow, or the increase in flow produced by exercise during normal arterial inflow, but does contribute to coronary vasodilation in hypoperfused myocardium. Therefore, we investigated whether adenosine opposed the hypoperfusion produced by K^+_{ATP} channel blockade, thereby contributing to coronary vasodilation during exercise. 11 dogs were studied at rest and during exercise under control conditions, during intracoronary infusion of the K^+_{ATP} channel blocker glibenclamide (50 μg/kg per min), and during intracoronary glibenclamide in the presence of adenosine receptor blockade. Glibenclamide decreased resting coronary blood flow from 45±5 to 35±4 ml/min ($P < 0.05$), but did not prevent exercise-induced increases of coronary flow. Glibenclamide caused an increase in myocardial oxygen extraction at the highest level of exercise with a decrease in coronary venous oxygen tension from 15.5±0.7 to 13.6±0.8 mmHg ($P < 0.05$). The addition of the adenosine receptor antagonist 8-phenyltheophylline (5 mg/kg intravenous) to K^+_{ATP} channel blockade did not further decrease resting coronary blood flow but did attenuate the increase in coronary flow produced by exercise. This was accompanied by a further decrease of coronary venous oxygen tension to 10.1±0.7 mmHg ($P < 0.05$), indicating aggravation of the mismatch between oxygen demand and supply. These findings are compatible with the hypothesis that K^+_{ATP} channels modulate coronary vasomotor tone both under resting conditions and during exercise. However, when K^+_{ATP} channels are blocked, adenosine released from the hypoperfused myocardium provides an alternate mechanism to mediate coronary vasodilation in response to increases in oxygen demand produced by exercise. (*J. Clin. Invest.* 1995. 95:285–295.) Key words: coronary blood flow • myocardial ischemia • myocardial oxygen consumption • regional myocardial systolic wall thickening

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Introduction

In the normal heart, coronary blood flow (CBF)[1] is regulated in response to changing metabolic needs to maintain a consistently high level of oxygen extraction by the myocardium. This close coupling between myocardial metabolic demands and CBF, which is especially apparent during exercise, has been suggested to depend on messengers released from the myocardium or vascular endothelium. However, selective blockers of established endogenous vasodilators such as adenosine (1), prostacyclin (2), and nitric oxide (3) have not been found to impair the normal increases in CBF that occur during exercise, indicating that these vasodilators are not mandatory for coronary vasodilation produced by exercise in the normal heart. In contrast, these vasodilator mechanisms can contribute to regulation of coronary vasomotor tone during exercise in the presence of myocardial ischemia. Thus, blockade of adenosine (4, 5) or nitric oxide (6) impaired coronary microvascular vasodilation distal to a coronary artery stenosis that resulted in myocardial hypoperfusion during exercise.

Recent evidence indicates that hyperpolarization of the vascular smooth muscle cell membrane caused by opening of K^+_{ATP} channels is an important mechanism for coronary dilation. Thus, blockade of vascular smooth muscle K^+_{ATP} channels decreased CBF at rest (7, 8) and blunted the increase in flow during reactive hyperemia (8, 9). Paradoxically, however, K^+_{ATP} channel blockade did not attenuate the increase in coronary flow produced by treadmill exercise (8). Myocardial adenosine content is increased in response to an increased cardiac workload (10–14) and to myocardial ischemia (15). Consequently, we hypothesized that the failure of K^+_{ATP} channel blockade to prevent the increase in CBF in response to exercise could be accounted for by augmented myocardial adenosine production. If this were true then adenosine receptor blockade should inhibit the exercise-induced coronary vasodilation which persists after K^+_{ATP} channel blockade. To test this hypothesis we investigated the effects of K^+_{ATP} channel blockade alone and in combination with adenosine receptor blockade on the increases in CBF produced by exercise.

Methods

Studies were performed in 16 adult mongrel dogs weighing 20–27 kg and trained to run on a motor-driven treadmill. All experiments were

1. *Abbreviations used in this paper:* CBF, coronary blood flow; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery.

performed in accordance with the Guiding Principles in the Care and Use of Laboratory Animals as approved by the Council of the American Physiological Society, and with the prior approval of the Animal Care Committee of the University of Minnesota.

Surgical preparation

After sedation with acepromazine (0.5 mg/kg, intramuscularly) dogs were anesthetized with sodium pentobarbital (30–35 mg/kg, intravenous), intubated and ventilated with a mixture of oxygen (30%) and room air (70%). Respiratory rate and tidal volume were set to keep arterial blood gases within physiologic limits. A left thoracotomy was performed through the fifth intercostal space and the heart was suspended in a pericardial cradle. A polyvinyl chloride catheter, 3.0 mm outer diameter and filled with heparinized saline, was inserted into the left internal thoracic artery and advanced into the ascending aorta. Similar catheters were introduced into the left atrium through the atrial appendage and the left ventricle through the apical dimple. A solid state micromanometer (model P5; Konigsberg Instruments Inc., Pasadena, CA) was also introduced into the left ventricle at the apex. Approximately 1.5 cm of the proximal left anterior descending coronary artery (LAD) was dissected free and a Doppler flow velocity probe (Craig Hartley, Houston, TX) was positioned around the artery. Immediately distal to the flow probe a hydraulic occluder was placed around the vessel. A silicone catheter (0.3 mm, inner diameter), bonded to a larger silicone catheter (1.6 mm inner diameter) was introduced into the LAD immediately distal to the hydraulic occluder. Two pairs of 5-MHz miniature piezoelectric crystals to measure myocardial wall thickening were implanted in the area perfused by the LAD and the left circumflex coronary artery (control area), respectively. In eight animals, a polyvinyl chloride catheter (3.0 mm outer diameter) was introduced into the right atrial appendage, manipulated into the coronary sinus ostium, and advanced 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. Subsequently, the catheter was secured with a purse string suture. 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 evacuated. Catheters were flushed daily with heparinized saline.

Hemodynamic measurements

Studies were performed 2–3 wk after surgery with the animals exercising on a motor-driven treadmill. Phasic and mean aortic pressure were measured with pressure transducers (P23XL; Gould Inc., Glen Burnie, MD) positioned at mid-chest level. Left ventricular pressure was measured with the micromanometer calibrated with the fluid-filled left ventricular catheter. The first derivative of left ventricular pressure (dP/dt) was obtained via electrical differentiation of the left ventricular pressure signal. Coronary blood velocity was measured with a Doppler flowmeter system (Craig Hartley). Data were recorded on an eight-channel direct writing oscillograph (Coulbourn Instruments Inc., Allentown, PA).

Regional myocardial function measurements

Regional myocardial wall thickening was measured by sonomicrometry (model 120; Triton Technology Inc., San Diego, CA) using two pairs of 5-MHz ultrasonic crystals. Reliable tracking of the crystals was not achieved in the left circumflex coronary artery control segment in one animal, and in the LAD segment of another. End-diastolic wall thickness (EDT) was measured at the onset of positive left ventricular dP/dt and end-systolic wall thickness (EST) was measured 20 ms before peak negative left ventricular dP/dt. Percent myocardial systolic wall thickening (SWT) was calculated as:

$$\text{SWT}\ (\%) = (\text{EST} - \text{EDT})/\text{EDT} \times 100$$

Experimental protocols

Magnitude and selectivity of K^+_{ATP} channel blockade. In five resting dogs the magnitude and selectivity of glibenclamide as a K^+_{ATP} channel blocker was assessed. For this purpose we measured the increases in CBF caused by the K^+_{ATP} channel opener pinacidil (0.25, 0.5, 1, and 2.5 μg/kg per min) infused directly into the coronary artery. After completion of these measurements, an infusion of glibenclamide was started into the coronary artery in a dose of 50 μg/kg per min at a rate of 1.5 ml/min. While the glibenclamide infusion was continued, the pinacidil infusions were repeated (0.5, 1, 2.5, and 5 μg/kg per min) and CBF measurements obtained. On separate days we studied the effects of glibenclamide on the increases in CBF produced by nitroprusside (0.6, 1.5, and 3.0 μg/kg per min) and adenosine (1, 2.5, 5, 10, 25, and 50 μg/kg per min). All drugs were dissolved in deionized water (pH 6.5–8.5).

Magnitude and selectivity of adenosine receptor blockade. In three resting dogs the magnitude and selectivity of adenosine receptor blockade produced by 8-phenyltheophylline was determined. For this purpose we measured the increases in CBF caused by intracoronary infusions of adenosine (0.5–5 μg/kg per min), nitroprusside (0.6–3.0 μg/kg per min) and pinacidil (0.25–2.5 μg/kg per min), infused in random order. After completion of these measurements, 8-phenyltheophylline was administered in a dose of 5 mg/kg, infused intravenously over a period of 5 min. 8-Phenyltheophylline was dissolved in deionized water (pH 10–11). 10 min after completion of drug administration, intracoronary infusions of adenosine (2.5–25 μg/kg per min), nitroprusside (0.6–3.0 μg/kg per min) and pinacidil (0.5–5 μg/kg per min) were repeated.

Reactive hyperemia protocol. On a different day, the effects of glibenclamide with and without 8-phenyltheophylline on the reactive hyperemic responses to coronary artery occlusions were studied in seven dogs. With dogs resting quietly in a sling, baseline measurements were made of systemic hemodynamics and CBF. Then, reactive hyperemic responses to coronary occlusions 5, 10, and 20 s in duration were recorded in duplicate. A 3-min interval was allowed between occlusions. Subsequently, an infusion of glibenclamide in a dose of 50 μg/kg per min was started into the coronary artery catheter at a rate of 1.5 ml/min. 5 min later, hemodynamic measurements were collected and the reactive hyperemic responses to coronary artery occlusions 5-, 10-, and 20-s in duration were obtained in the presence of the glibenclamide infusion. Then the glibenclamide infusion was discontinued and adenosine receptor blockade was produced with 8-phenyltheophylline in a dose of 5 mg/kg infused intravenously over 5 min. 10 min after completion of the 8-phenyltheophylline infusion, the glibenclamide infusion was restarted in a dose of 50 μg/kg per min. 5 min later, hemodynamic measurements were collected and the reactive hyperemic responses to coronary artery occlusions 5-, 10-, and 20-s in duration were repeated in the presence of adenosine receptor blockade and glibenclamide infusion.

Exercise protocol. On a different day, 11 animals underwent a graded treadmill exercise protocol. With the dogs standing quietly on the treadmill, resting hemodynamic and regional wall function measurements were obtained and arterial and coronary venous blood samples collected. Subsequently, a four-stage treadmill exercise protocol was begun (4.8 km/h at 0% grade, 6.4 km/h at 0% grade, 6.4 km/h at 10% grade, 6.4 km/h at 20% grade). Each exercise stage was ~ 2 min in duration; left ventricular and aortic blood pressure, CBF and myocardial function were measured and blood samples collected during the last 30 s of each exercise stage, when hemodynamics had reached a steady state.

After 90 min of rest an infusion of glibenclamide (50 μg/kg per min) was begun into the coronary artery catheter at a rate of 1.5 ml/min. 5 min after beginning the infusion, resting measurements were obtained and the four-stage exercise protocol repeated during the glibenclamide infusion. After completion of the exercise protocol the glibenclamide infusion was discontinued and the animals allowed to rest for 90 min. Then adenosine blockade was produced by infusing 8-phenyltheophylline in a dose of 5 mg/kg over 5 min. 10 min later the intracoronary infusion of glibenclamide (50 μg/kg per min) was restarted; 5 min after beginning the glibenclamide infusion the exercise protocol was repeated.

Blood specimens were maintained in iced syringes until the conclusion of each exercise protocol. Measurements of P_{O_2}, P_{CO_2}, and pH

were then immediately performed with a blood gas analyzer (model 113; Instrumentation Laboratory, Lexington, MA). Hemoglobin content was determined with the cyanomethemoglobin method. Hemoglobin saturation was estimated from the blood Po_2, pH, and temperature using the oxygen dissociation curve for canine blood (16). Blood oxygen content was computed as (Hb × 1.34 × % O_2 saturation) + (0.0031 × Po_2). Oxygen consumption in the region of myocardium perfused by the LAD was calculated as the product of blood flow measured with the Doppler flow probe and the difference in oxygen content between aortic and coronary venous blood.

Data analysis

Heart rate, left ventricular and aortic pressures, CBF and regional wall thickness of the LAD-perfused area and the control area were measured from the strip chart recordings CBF was computed from the Doppler shift using the equation $Q = 2.5 \cdot \Delta f \cdot d^2$, where Q is the CBF (ml/min), Δf is the Doppler shift (KHz), and d is the internal diameter of the coronary artery (mm) within the flow probe (17). The factor 2.5 is a constant derived from the speed of sound in tissue ($C = 1.5 \cdot 10^5$ cm/s), the frequency of the emitted sound beam (f_0 = 10 MHz), the cosine of the angle at which the sound beam is emitted (45°), and unit conversion factors: $(C \cdot \pi/4 \cdot 3)/(2f_0 \cdot \cos 45°)$ (17). Since, in chronically instrumented animals, the flow probe is tightly adherent to the coronary artery, the internal diameter of the flow probe is equal to the external diameter of the artery. To obtain the inner diameter of the coronary artery we subtracted the arterial wall thickness which, in our experience, is ~ 20% of the external diameter of the coronary artery. In this way any error in computation of the coronary internal diameter would affect control and intervention conditions equally.

Total blood flow during reactive hyperemia was determined by electrical integration of the Doppler shift tracing. Reactive hyperemia flow was calculated as: reactive hyperemia excess flow = total flow during reactive hyperemia (ml) − [control flow rate (ml/s) × duration of reactive hyperemia (s)]. In the exercise protocol, CBF was analyzed as a function of heart rate, the product of heart rate and left ventricular systolic pressure, and a calculated index of time-averaged systolic wall stress. The index of wall stress was computed as the product of heart rate (HR) and left ventricular systolic pressure (LVSP) divided by the average systolic wall thickness. The latter was estimated by calculating median systolic wall thickness from the end-diastolic thickness (EDT) and end-systolic thickness (EST) as (EDT + EST)/2. Time-averaged systolic wall stress was then computed as: (HR·LVSP)/[(EDT + EST)/2].

Statistical analysis was performed using two-way (exercise level and treatment) ANOVA for repeated measures. When a significant effect of exercise was observed, comparisons within treatment groups were made using one-way ANOVA followed by Scheffe's post-hoc test. When a significant difference between treatments was observed, comparisons between groups were made with the Wilcoxon signed rank test. The effect of treatment on the relationship between two variables was analyzed by multiple stepwise regression analysis. Statistical significance was accepted at $P < 0.05$ (two-tailed). All data are presented as mean±SEM.

Results

Magnitude and selectivity of K^+_{ATP} channel blockade by glibenclamide

Intracoronary infusions of adenosine had no effect on heart rate (110±11 bpm) or mean aortic pressure (96±5 mmHg). Pinacidil infusions caused a small increase in heart rate from 103±5 bpm during control to 112±5 bpm at the highest dose ($P < 0.05$), with no significant effect on mean aortic pressure (93±3 mmHg). Sodium nitroprusside decreased mean aortic pressure from 101±6 mmHg to 89±6 mmHg ($P < 0.05$) and tended to increase heart rate from 104±8 to 120±12 bpm (NS).

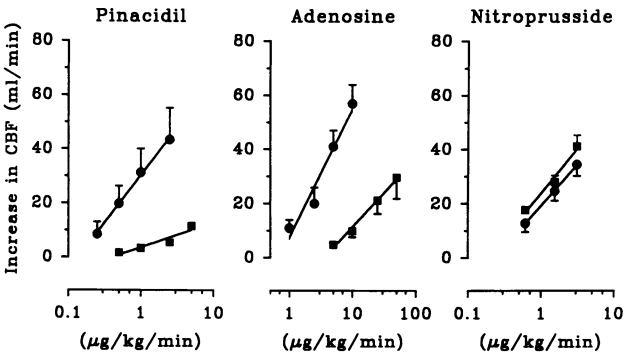

Figure 1. Effects of K^+_{ATP} channel blockade on increases in CBF from baseline produced by intracoronary infusions of pinacidil, adenosine, and nitroprusside in five awake resting dogs. Shown are the dose-response curves under control conditions (*circles*) and during infusion of glibenclamide (50 μg/kg per min, intracoronary) (*squares*). Data are mean±SEM.

Glibenclamide did not significantly alter the systemic responses to adenosine and sodium nitroprusside, but the slight increase in heart rate produced by pinacidil under control conditions was absent in the presence of the K^+_{ATP} channel blocker.

The effects of glibenclamide on the increments in CBF produced by intracoronary infusions of pinacidil, adenosine, and nitroprusside are shown in Fig. 1. Glibenclamide had no effect on the increase in coronary flow caused by nitroprusside but markedly decreased the coronary vasodilation caused by pinacidil with 85±5% inhibition of the increase in coronary flow produced by a pinacidil dose of 2.5 μg/kg per min. The CBF responses to adenosine were also markedly inhibited, although slightly less than the pinacidil-induced hyperemia. Accordingly, a 10-fold higher dose of adenosine and an ~ 15-fold higher dose of pinacidil were required to elicit a 10 ml/min increase in CBF in the presence of glibenclamide compared with control conditions (Fig. 1).

Magnitude and selectivity of adenosine receptor blockade by 8-phenyltheophylline

With dogs standing in a sling, 8-phenyltheophylline had no effect on mean aortic blood pressure (99±6 mmHg) but tended to increase heart rate from 125±14 to 135±18 bpm which was not statistically significant. 8-Phenyltheophylline did not significantly alter the systemic responses to pinacidil, adenosine, or nitroprusside. The increases in CBF produced by intracoronary infusions of pinacidil and nitroprusside were also not altered by 8-phenyltheophylline, but those produced by adenosine were markedly attenuated (Fig. 2).

Reactive hyperemia

During resting conditions heart rate and mean aortic blood pressure were 112±10 bpm and 103±7 mmHg, respectively. These measurements were not significantly altered by intracoronary infusion of glibenclamide (113±10 bpm and 103±7 mmHg), while 8-phenyltheophylline caused a slight increase in mean aortic pressure to 116±6 mmHg ($P < 0.05$) with no change in heart rate (119±9 bpm). An example of a reactive hyperemia response to a 10 second coronary artery occlusion is shown in Fig. 3. Glibenclamide decreased resting CBF, decreased peak blood flow rates during reactive hyperemia, and shortened the duration of reactive hyperemia following 5-, 10-, and 20-s oc-

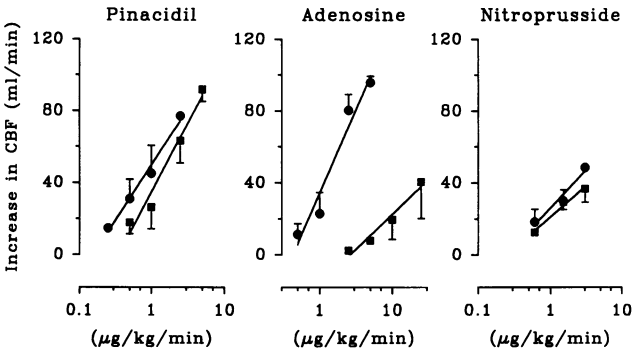

Figure 2. Effects of adenosine receptor blockade on increases in CBF from baseline produced by intracoronary infusions of pinacidil, adenosine and nitroprusside in three awake resting dogs. Shown are the increases in coronary flow under control conditions (*circles*) and in the presence of 8-phenyltheophylline (5 mg/kg, intravenous) (*squares*). Data are mean±SEM.

clusions. As a result, the reactive hyperemia excess flow was markedly attenuated by glibenclamide (Table I). The addition of 8-phenyltheophylline to glibenclamide did not further decrease resting CBF, but caused a decrease in peak blood flow rates during reactive hyperemia which reached levels of statistical significance after the 20-s occlusion. Since the duration of the reactive hyperemia tended to increase after the addition of adenosine blockade, the tendency for excess reactive hyperemic flow volume to decrease further did not reach statistical significance (Table I).

Exercise

Systemic hemodynamics. The hemodynamic responses to increasing levels of exercise are shown in Table II. Exercise increased heart rate from 115±6 at rest to a maximum of 245±9 bpm ($P < 0.01$), mean arterial pressure from 99±4 to 123±6 mmHg ($P < 0.01$), left ventricular systolic pressure from 123±5 to 162±5 mmHg ($P < 0.01$), and $LVdP/dt_{max}$ from 2,530±110 to 6,700±570 mmHg per s ($P < 0.01$), but did not significantly alter left ventricular end-diastolic pressure (Table II). Glibenclamide had no effect on any of the systemic hemodynamic variables at rest. During exercise, glibenclamide caused no change in heart rate and mean aortic pressure. However, glibenclamide caused decreases in left ventricular systolic pressure and $LVdP/dt_{max}$, which were accompanied by significant elevations of left ventricular end-diastolic pressure. With the addition of adenosine blockade, resting heart rate and aortic and left ventricular blood pressures tended to be higher compared to glibenclamide alone, but no significant changes in systemic hemodynamic variables were observed at rest or during exercise (Table II).

Coronary hemodynamics. The coronary hemodynamic responses to exercise are shown in Table III. Myocardial oxygen consumption increased from 5.6±0.6 ml O_2 per min at rest to 13.9±1.6 ml O_2 per min at the highest level of exercise (P

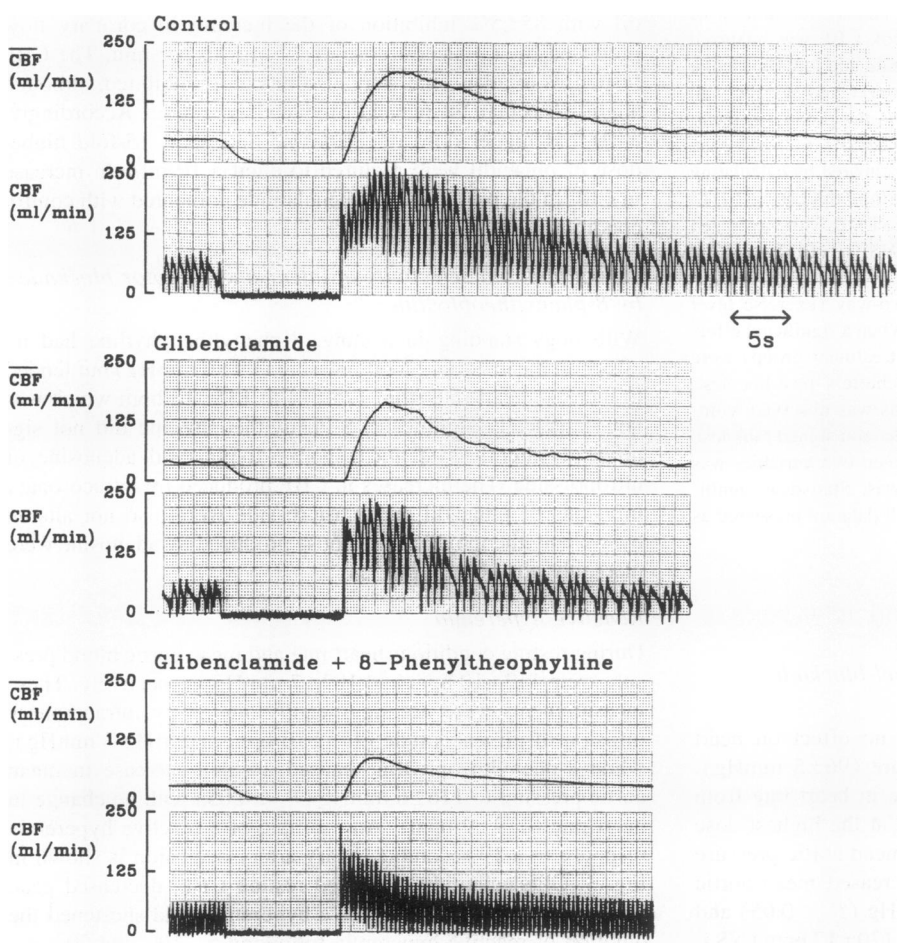

Figure 3. Example of a reactive hyperemic response which occurs in response to a 10-s coronary artery occlusion under control conditions, in the presence of K^+_{ATP} channel blockade with intracoronary glibenclamide, 50 μg/kg per min, and in the presence of K^+_{ATP} channel blockade with intracoronary glibenclamide, 50 μg/kg per min, and adenosine receptor blockade with 8-phenyltheophylline, 5 mg/kg intravenous.

Table I. Reactive Hyperemia after Occlusions of Left Anterior Descending Coronary Artery of 5-, 10-, and 20-s Duration in Seven Dogs

	Occlusion duration								
	5-s			10-s			20-s		
	Con	G	8PT+G	Con	G	8PT+G	Con	G	8PT+G
CBF basal (ml/min)	49±5	33±3*	34±5*	52±6	34±2*	34±6*	52±6	32±2*	35±4*
CBF peak (ml/min)	146±14	89±7*	84±13*	177±18	115±11*	96±13*	179±20	129±13*	103±11*‡
CVC peak (ml/min per mmHg)	1.39±0.16	0.87±0.08*	0.74±0.12*	1.71±0.24	1.09±0.10*	0.83±0.10*‡	1.73±0.25	1.20±0.09*	0.88±0.09*‡
Duration (s)	18.4±3.3	11.0±1.3*	18.5±2.1‡	28±5	20±2	27±3	46±8	28±3	35±4
Reactive hyperemia excess flow (ml)	9.8±2.6	4.2±0.7*	5.2±1.4	20.6±3.3	10.3±1.8*	8.1±1.6*	36±8	17±4*	14±3*

Values are mean±SEM. Con, control; G, glibenclamide 50 μg/kg per min intracoronary; 8PT, 8-phenyltheophylline 5 mg/kg intravenous; CVC, coronary vascular conductance (flow/pressure); duration = time from release of the occluder to recovery of CBF to basal level; reactive hyperemia excess flow, total reactive hyperemia blood flow − (basal flow rate × duration of reactive hyperemia). * $P < 0.05$ vs corresponding control measurement, ‡ $P < 0.05$ vs corresponding glibenclamide measurement.

< 0.01). The increase in oxygen consumption was accounted for by an increase in CBF from 45±5 ml/min at rest to 92±10 ml/min at the highest level of exercise ($P < 0.01$), and an increase in the arterial-coronary venous oxygen content difference from 12.1±0.4 ml O_2 per 100 ml to 14.9±0.7 ml O_2 per 100 ml ($P < 0.01$). The increase in myocardial oxygen extraction from 76±2% at rest to 83±2% during exercise ($P < 0.01$) caused a decrease in coronary venous oxygen tension from 18.8±1.0 Torr at rest to 15.5±0.7 Torr during peak exercise ($P < 0.05$). In the presence of glibenclamide, CBF was significantly lower at rest and during each level of exercise (Table III), so that glibenclamide caused a parallel downward shift in the relationship between either one of three indices of myocardial oxygen demand and CBF (Fig. 4). Thus, the slope of the relationship between CBF and heart rate was not altered by glibenclamide (36.1±5.1 10^{-2} ml during control versus 28.2±5.0 10^{-2}·ml during glibenclamide; P = NS). Similarly, the slope of the relationship between CBF and the product of heart rate and left ventricular systolic pressure (18.4±2.5 10^{-4}·ml/mmHg during control vs 15.0±2.4 10^{-4}·ml/mmHg during glibenclamide; P = NS), and the slope of the relationship between CBF and an index of systolic wall stress (15.4±2.2 10^{-3}·ml·mm/mmHg during control vs 11.9±2.2 10^{-3}·ml·mm/mmHg per min during glibenclamide; P = NS), were also not significantly altered by glibenclamide (Fig. 4). The decrease in CBF was accompanied by a widening of the arterial coronary-venous oxygen content difference and an increase in oxygen extraction (Table III). Glibenclamide caused a downward shift of the relationship between myocardial oxygen consumption and coronary-venous oxygen tension (Fig. 5), indicating impairment of the myocardial oxygen supply. During combined adenosine receptor and K^+_{ATP} channel blockade CBF was significantly decreased compared to K^+_{ATP} channel blockade alone (Table III; Fig. 4). The small increase in CBF in response to exercise was mainly the result of an increase in mean aortic pressure, as the increase in computed coronary vascular conductance failed to reach statistical significance (P = 0.07). The slope of the relationship between CBF and heart rate was decreased by the addition of adenosine receptor blockade (from 28.2±5.0 10^{-2}·ml to 15.3±5.9 10^{-2}·ml, $P < 0.01$). Similarly, the slope of the relationship between CBF and the product of heart rate and left ventricular systolic pressure decreased from 15.0±2.4 10^{-4}·ml/mmHg to 7.9±3.3 10^{-4}·ml/mmHg, $P < 0.02$), and the slope of the relationship between CBF and an index of systolic wall stress also decreased, from 11.9±2.2 10^{-3}·ml·mm/mmHg per min to 4.8±3.1 10^{-3}·ml·mm/mmHg per min, $P < 0.01$). Myocardial oxygen extraction was further increased with the addition of 8-phenyltheophylline to glibenclamide, so that for each level of myocardial oxygen consumption, coronary venous oxygen tension was significantly further reduced (Fig. 5).

Regional myocardial contractile function. Exercise had no effect on end-diastolic myocardial wall thickness but increased end-systolic wall thickness in both the LAD perfused area (Table IV) and the left circumflex coronary artery (LCX) perfused control region (Table V). As a result, systolic wall thickening in the LAD perfused region increased from 20±2% at rest to 25±2% ($P < 0.05$), and thickening in the LCX perfused region increased from 18±2% at rest to 25±2% ($P < 0.05$). Glibenclamide had no effect on the end-diastolic wall thickness but decreased end-systolic thickness of the LAD perfused region at rest and during each level of exercise. Percent systolic wall thickening was significantly reduced by glibenclamide so that at the highest level of exercise, the thickening fraction was 18±2% ($P < 0.05$). During combined adenosine receptor and K^+_{ATP} channel blockade, systolic wall thickening in the anterior wall was further decreased at rest and during exercise compared to K^+_{ATP} channel blockade alone, so that at the highest level of exercise wall thickening was 8% ($P < 0.05$ vs glibenclamide alone). Systolic wall thickening in the posterior region perfused by the LCX was not different from exercise under control conditions.

Discussion

This study is the first to document a synergistic relationship between K^+_{ATP} channel activation and endogenous adenosine for the coronary vasodilation which occurs in response to the increased myocardial metabolic demands produced by exercise. Glibenclamide decreased resting CBF but did not attenuate the

Table II. Hemodynamic Data at Rest and during Graded Treadmill Exercise

	Heart rate			Mean aortic pressure			LV Systolic pressure			End diastolic pressure			$LVdP/dt_{max}$		
	Con	G	8PT+G	Con	G	8PT+G	Con	G	8PT+G	Con	G	8PT+G	Con	G	8PT+G
		bpm			*mmHg*			*mmHg*			*mmHg*			*mmHg/s*	
Rest	115±6	131±7	143±7‡	99±4	103±4	111±5‡	123±5	123±5	128±5	7.0±0.9	10.1±2.4	11.8±2.1‡	2,530±110	2,520±180	2,650±180
Exercise (speed/grade)															
4.8 km/h, 0%	175±9*	177±7*	172±8*	110±5*	110±5	116±6	137±5*	134±5*	136±6*	9.1±1.2	13.6±2.6‡	15.2±1.9‡	3,850±250*	3,400±250*‡	3,470±260*
6.4 km/h, 0%	202±9*	204±8*	198±9*	112±6*	112±7*	118±6	143±6*	138±6*	139±5*	10.1±1.5	13.5±2.5‡	17.4±1.8*‡	4,450±300*	4,050±350*	4,140±370*
6.4 km/h, 10%	220±9*	219±8*	211±9*	113±6*	113±7*	118±6	147±6*	141±6*‡	142±5*	9.5±1.7	13.1±2.4	15.8±1.8‡	5,070±440*	4,590±410*	4,470±410*‡
6.4 km/h, 20%	245±9*	244±8*	239±9*	123±6*	120±7*	122±6*	162±5*	152±6*‡	149±5*‡	10.6±1.8	13.3±1.9‡	16.3±2.1‡	6,700±570*	5,880±520*‡	5,490±520*‡

LV, left ventricular; Con, control; G, glibenclamide 50 μg/kg per min intracoronary; 8PT, 8-phenyltheophylline 5 mg/kg intravenous. Values are mean±SEM. $n = 11$; * $P < 0.05$ vs rest, ‡ $P < 0.05$ vs corresponding control measurements, § $P < 0.05$ vs corresponding glibenclamide measurements.

Table III. Coronary Hemodynamic Data at Rest and during Graded Treadmill Exercise

	CBF			Coronary vascular conductance			Arterial-coronary venous O_2 content difference			Myocardial O_2 consumption			Myocardial O_2 extraction		
	Con	G	8PT+G	Con	G	8PT+G	Con	G	8PT+G	Con	G	8PT+G	Con	G	8PT+G
		ml/min			*10^{-2}·(ml/min per mmHg)*			*vol%**			*ml/min**			*%**	
Rest	45±5	35±4‡	35±5‡	46±4	34±4	31±5‡	12.1±0.4	13.2±0.7‡	14.1±0.6‡§	5.6±0.6	4.1±0.5‡	4.6±0.7‡	76±2	82±2‡	87±2‡§
Exercise (speed/grade)															
4.8 km/h, 0%	70±7‖	49±5‡	41±6‡	63±5‖	44±4‖‡	36±5‡	12.9±0.5	13.7±0.6‡	14.4±0.5‡§	9.0±0.9‖	6.1±0.7‡	5.8±1.0‡	81±1‖	86±1‡	89±1‡§
6.4 km/h, 0%	79±8‖	55±6‡	45±7‡	70±6‖	50±5‖‡	38±6‡	13.5±0.5‖	14.3±0.7‖	15.2±0.5‡§	10.8±1.4‖	7.5±0.9‖‡	7.0±1.3‡	82±1‖	86±2	91±1‖‡§
6.4 km/h, 10%	83±9‖	60±6‖‡	46±7‡§	73±6‖	52±5‖‡	38±6‡§	14.1±0.6‖	15.2±0.7‖‡	15.8±0.6‖‡	11.8±1.4‖	8.7±1.1‖‡	7.5±1.5‖‡	82±1‖	89±1‖‡	92±1‖‡
6.4 km/h, 20%	92±10‖	68±8‖‡	49±8‖‡§	74±7‖	56±5‖‡	40±6‡§	14.9±0.7‖	15.6±0.8‖‡	16.6±0.6‖‡§	13.9±1.6‖	10.5±1.4‖‡	8.8±1.7‖‡	83±2‖	87±1‖‡	92±1‖‡§

Con, control; G, glibenclamide 50 μg/kg per min intracoronary; 8PT, 8-phenyltheophylline 5 mg/kg intravenous. Values are mean±SEM; $n = 11$, * $n = 8$; ‖ $P < 0.05$ vs rest, ‡ $P < 0.05$ vs corresponding control measurements, § $P < 0.05$ vs corresponding gilbenclamide measurements.

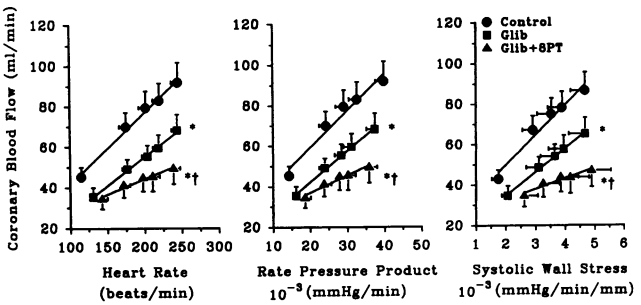

Figure 4. Relationship between heart rate and CBF (*left panel, n* = 11), the product of heart rate, left ventricular systolic pressure (rate pressure product) and CBF (*middle panel, n* = 11), and between an index of systolic wall stress and CBF (*right panel, n* = 10) in dogs at rest and during incremental levels of treadmill exercise. Shown are the relationships under control conditions (*circles*), in the presence of K^+_{ATP} channel blockade with intracoronary glibenclamide, 50 μg/kg per min (*squares*), and in the presence of K^+_{ATP} channel blockade with intracoronary glibenclamide, 50 μg/kg per min, and adenosine receptor blockade with 8-phenyltheophylline, 5 mg/kg intravenous (*triangles*). Data are mean±SEM; $*P < 0.05$ vs control; $^{\dagger}P < 0.05$ vs glibenclamide.

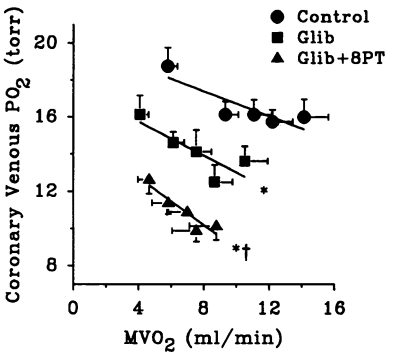

Figure 5. Relationship between myocardial oxygen consumption (MVO_2) and coronary venous oxygen tension (coronary venous P_{O_2}) in eight dogs at rest and during incremental levels of treadmill exercise. Shown are the relationships under control conditions (*circles*), in the presence of K^+_{ATP} channel blockade with intracoronary glibenclamide, 50 μg/kg per min (*squares*), and in the presence of K^+_{ATP} channel blockade with intracoronary glibenclamide, 50 μg/kg per min, and adenosine receptor blockade with 8-phenyltheophylline, 5 mg/kg intravenous (*triangles*). Data are mean±SEM; $*P < 0.05$ vs control; $^{\dagger}P < 0.05$ vs glibenclamide.

exercise-induced increase in CBF. The addition of adenosine receptor blockade, which under normal conditions has no effect on CBF at rest or during exercise (1), did not further decrease resting CBF, but markedly attenuated the exercise-induced coronary vasodilation which persisted during K^+_{ATP} channel blockade alone. These findings suggest that K^+_{ATP} channels normally do contribute to coronary vasodilation during exercise, but that in the presence of K^+_{ATP} channel blockade, increased endogenous adenosine production provides an alternate mechanism for coronary vasodilation produced by exercise.

Coronary blood flow at rest. Previous studies have demonstrated that endogenous adenosine production is not obligatory for maintaining resting CBF. Thus, studies in anesthetized open-chest dogs failed to demonstrate an effect of intracoronary adenosine deaminase (18–20) or intravenous aminophylline to block adenosine receptors (21, 22), on basal CBF. Similarly, in awake dogs intracoronary adenosine deaminase or intravenous 8-phenyltheophylline, in doses that caused marked inhibition of exogenous adenosine-induced coronary vasodilation, had no effect on resting CBF (1, 23).

Several studies in anesthetized and awake dogs have investigated the role of K^+_{ATP} channels in maintaining resting CBF. Using extracorporeally perfused canine hearts, Aversano et al. (9) found that intracoronary glibenclamide in doses of 0.8 and 3.7 μmol/min had no effect on basal CBF. Doses of glibenclamide of 0.8 and 3.7 μmol/min administered into the coronary artery of 25–30-kg dogs correspond to intracoronary infusions of ~ 14.4 and 66.5 μg/kg per min ($MW_{glibenclamide}$ = 494), which are in the dose range used in the present study. In contrast, Imamura et al. (24) reported that intracoronary glibenclamide in a dose of 50 μg/kg per min caused a 55% decrease in basal coronary blood in open-chest dogs. Similar to the latter study (8) we observed that glibenclamide in a dose of 50 μg/kg per min caused a 20–30% decrease in basal CBF in awake dogs.

Studies in anesthetized (24) and awake dogs (8) reported that the decrease in CBF produced by K^+_{ATP} channel blockade with glibenclamide was associated with a decrease in regional systolic wall thickening. When CBF was restored to pre-glibenclamide levels with nitroprusside (which itself was devoid of any direct effect on systolic wall thickening) contractile performance recovered as well (8, 24), suggesting that glibenclamide caused a primary decrease in coronary flow with a secondary decrease in contractile function. In addition, the decrease in CBF caused by glibenclamide caused metabolic changes suggestive of ischemia, including a decrease in phosphorylation potential with a decrease of myocardial creatine phosphate and

Table IV. Regional Myocardial Contractile Function of the Anterior Region at Rest and during Graded Treadmill Exercise

	End-diastolic thickness			End-systolic thickness			Systolic wall thickening		
	Con	G	8PT+G	Con	G	8PT+G	Con	G	8PT+G
		mm			*mm*			%	
Rest	7.8±0.5	7.8±0.5	7.6±0.5*‡	9.3±0.6	8.6±0.6*	8.1±0.6*‡	20±2	11±2*	7±2*
Exercise (speed/grade)									
4.8 km/h, 0%	7.8±0.6	7.8±0.5	7.7±0.6*	9.6±0.6	8.8±0.6*	8.3±0.7*‡	23±2	14±2*	8±1*‡
6.4 km/h, 0%	7.7±0.6	7.7±0.6	7.7±0.6	9.6±0.7	8.9±0.6*	8.3±0.7*‡	25±2§	15±2§*	7±1*‡
6.4 km/h, 10%	7.8±0.6	7.7±0.6	7.6±0.6*	9.8±0.7§	8.9±0.7*	8.2±0.7*‡	26±2§	16±2§*	7±2*‡
6.4 km/h, 20%	7.9±0.6	7.8±0.6	7.6±0.6*‡	9.9±0.7§	9.1±0.7§*	8.3±0.7*‡	25±2§	18±2§*	8±2*‡

Con, control; G, glibenclamide 50 μg/kg per min intracoronary; 8PT, 8-phenyltheophylline 5 mg/kg intravenous. Values are mean±SEM; n = 10, § $P < 0.05$ vs rest, * $P < 0.05$ vs corresponding control measurements, ‡ $P < 0.05$ vs corresponding gilbenclamide measurements.

Table V. Regional Myocardial Contractile Function of the Posterior Control Region at Rest and during Graded Treadmill Exercise

	End-diastolic thickness			End-systolic thickness			Systolic wall thickening		
	Con	G	8PT+G	Con	G	8PT+G	Con	G	8PT+G
		mm			*mm*			%	
Rest	8.9±0.4	8.9±0.4	8.7±0.4	10.4±0.4	10.4±0.5	10.5±0.4	18±2	18±2	21±2*‡
Exercise (speed/grade)									
4.8 km/h, 0%	9.0+0.4	8.9±0.4	8.7±0.8*‡	10.8±0.4§	10.6±0.4	10.6±0.4	21±2	20±2	22±2
6.4 km/h, 0%	8.9±0.4	8.9±0.4	8.7±0.4*‡	10.9±0.4§	10.8±0.5§	10.7±0.4	22±2§	22±2§	23±2
6.4 km/h, 10%	9.0±0.4	8.9±0.4	8.7±0.4*‡	11.1±0.5§	10.9±0.4§*	10.8±0.4*	24±2§	23±2§	24±2§
6.4 km/h, 20%	9.1±0.4§	8.9±0.4*	8.8±0.4*	11.4±0.5§	11.1±0.4§*	11.0±0.4§*	25±2§	25±2§	25±2§

Con, control; G, glibenclamide 50 μg/kg per min intracoronary; 8PT, 8-phenyltheophylline 5 mg/kg intravenous. Values are mean±SEM; $n = 10$, § $P < 0.05$ vs rest, * $P < 0.05$ vs corresponding control measurements, ‡ $P < 0.05$ vs corresponding glibenclamide measurements.

an increase of inorganic phosphate in open-chest dogs (7). Thus, both mechanical and metabolic evidence support the concept that K^+_{ATP} channel blockade produced coronary vasoconstriction which resulted in ischemia-induced contractile dysfunction.

In the present study we observed that the addition of adenosine receptor blockade decreased coronary venous oxygen tension and increased oxygen extraction compared to K^+_{ATP} channel blockade alone. In addition, systolic wall thickening tended to decrease with the addition of adenosine receptor blockade, suggesting that the myocardial oxygen supply-demand balance slightly further deteriorated. Since CBF was maintained it is likely that increased myocardial oxygen demand was produced by the small increases in heart rate and left ventricular systolic pressure after 8-phenyltheophylline.

Coronary blood flow during exercise. To examine the relationship between tissue adenosine content and CBF at different cardiac workloads, McKenzie et al. (12) obtained myocardial biopsies during treadmill exercise in chronically instrumented dogs. Exercise resulted in an increase in myocardial adenosine content from 1.35±0.54 to 8.18±0.60 nmol/g. Arterial and coronary sinus plasma levels of adenosine were similar at rest; exercise had no effect on arterial adenosine but nearly doubled the coronary venous concentration. Thus, increased myocardial oxygen demands resulted in increased myocardial adenosine production with significant spillover into the coronary venous blood, suggesting increased interstitial periarteriolar adenosine concentrations. Several investigators sampled pericardial fluid to estimate myocardial interstitial fluid adenosine concentration. In dogs with chronically implanted pericardial catheters, graded exercise was associated with progressive increases of pericardial infusate adenosine concentration (10, 11, 13), with a positive correlation between adenosine concentrations and CBF. Although these findings indicate that myocardial adenosine production increases during exercise, demonstration of an essential role for adenosine requires that interruption of the adenosine effect interfere with exercise-induced coronary vasodilation. Bache et al. (1) examined the effect of adenosine receptor blockade with 8-phenyltheophylline and increased adenosine catabolism with adenosine deaminase on exercise-induced increases in CBF. Neither agent caused a significant change of the response of heart rate, arterial pressure, or myocardial oxygen consumption to graded treadmill exercise. CBF responses to exercise were unaltered. Moreover, the relationship between myocardial oxygen consumption and CBF or coronary venous oxygen tension was not changed. Edlund et al. (25) examined the effect of adenosine receptor blockade with theophylline on the increase in coronary sinus blood flow measured with the thermodilution technique during supine exercise in normal young human subjects. Theophylline increased coronary vascular resistance at rest and during exercise, but the decrease in coronary resistance in response to exercise was not altered. These findings indicate that adenosine is not obligatory for the increase in CBF produced by exercise.

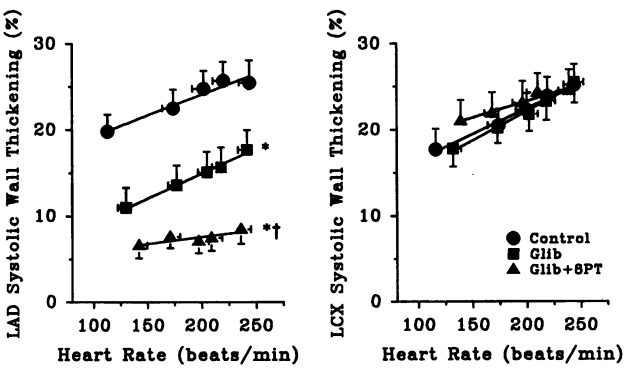

Figure 6. Relationship between heart rate and regional myocardial systolic wall thickening in the anterior wall, perfused by the left anterior descending coronary artery (LAD) (*left panel*, $n = 10$), and heart rate and regional myocardial systolic wall thickening in the posterior wall perfused by the left circumflex coronary coronary artery (LCX) (*right panel*, $n = 10$) in dogs at rest and during incremental levels of treadmill exercise. Shown are the relationships under control conditions (*circles*), in the presence of K^+_{ATP} channel blockade with intracoronary glibenclamide, 50 μg/kg per min (*squares*), and in the presence of K^+_{ATP} channel blockade with intracoronary glibenclamide, 50 μg/kg per min, and adenosine receptor blockade with 8-phenyltheophylline, 5 mg/kg intravenous (*triangles*). Data are mean±SEM; * $P < 0.05$ vs control; † $P < 0.05$ vs glibenclamide.

Previously (8) we reported that despite the K^+_{ATP} channel blockade-induced decrease in resting CBF, the increments in flow and contractile function produced by exercise were not attenuated by blockade of the K^+_{ATP} channels. This finding could be interpreted to indicate that K^+_{ATP} channels maintain a basal level of activity that inhibits coronary tone during resting conditions, but they are not essential for coronary vasodilation which

occurs during increments in myocardial oxygen demand produced by exercise. An alternative explanation is that when glibenclamide inhibites activation of K^+_{ATP} channels, other vasodilator systems are activated in response to further deterioration of the myocardial oxygen supply-demand balance that occurs during exercise. This concept is supported by observations in open-chest dogs by Samaha et al. (7) who reported that the addition of adenosine receptor blockade caused further deterioration of CBF and myocardial phosphorylation potential, compared to K^+_{ATP} channel blockade alone. This suggests that endogenous adenosine released from the myocardium can oppose the coronary vasoconstrictor effect of glibenclamide.

In the present study, adenosine opposed the hypoperfusion produced by K^+_{ATP} channel blockade during exercise. Thus, after K^+_{ATP} channel blockade had been established, the subsequent addition of adenosine blockade caused a further decrease in CBF accompanied by an increase in oxygen extraction and decreases in coronary venous oxygen tension and myocardial contractile performance. The effect of adenosine blockade on blood flow and contractile function was most pronounced during the higher levels of exercise. This suggests that after K^+_{ATP} channel blockade, increasing levels of exercise caused progressive deterioration of the oxygen supply-demand balance, thereby augmenting the release of adenosine into the myocardial interstitial space. This hypothesis is supported by the findings of Berne and co-workers (15) who observed progressively greater increases in interstitial adenosine concentrations in isovolumically beating hearts (myocardial oxygen consumption 73 μl/min per g) than in empty beating hearts (myocardial oxygen consumption 51 μl/min per g) when both underwent similar CBF reductions. Thus, epicardial interstitial adenosine concentrations increased from 165 to 300 nm in empty beating hearts when flow was reduced from 5.2 to 1.0 ml/min per g, while in isovolumically beating hearts adenosine concentrations increased from 175 to 600 nm when flow was reduced from 7.2 to 2.1 ml/min per g. Moreover, these authors observed excellent inverse correlations between the myocardial oxygen supply-demand balance and interstitial adenosine concentrations (15), and between the coronary venous oxygen tension and interstitial adenosine concentration (14). Therefore, it is likely that in the present study, impaired coronary vasodilation after K^+_{ATP} channel blockade caused progressive deterioration of the oxygen supply-demand balance with increasing exercise intensity. The consequent augmented release of adenosine into the myocardial interstitial space provided an alternate pathway for coronary vasodilation in response to exercise.

Reactive hyperemia. Previous studies have demonstrated that both adenosine and K^+_{ATP} channel blockade alter the coronary reactive hyperemic response. Thus, studies in anesthetized open-chest dogs have reported a decrease of the total volume of excess flow during reactive hyperemia (repayment area), after intracoronary adenosine deaminase (18), or intravenous aminophylline (21, 22) to block adenosine receptors, although there was no effect on peak reactive hyperemia flow. Similarly, in awake dogs adenosine deaminase or 8-phenyltheophylline, in doses that caused marked inhibition of exogenous adenosine-induced coronary vasodilation, had no effect on peak reactive hyperemia blood flow, but attenuated the late phase of the reactive hyperemia with marked shortening of the duration of the total response (1). Using stroboscopic epiillumination to visualize the subepicardial coronary microvasculature in open-chest dogs, Kanatsuka et al. (26) reported that 8-phenyltheophylline did not alter vasodilation of coronary arterioles (< 100 μm) in response to 30-s arterial occlusions, but decreased the time for return to baseline vessel diameter during subsequent reperfusion. These studies indicate that adenosine is not essential for the microvascular vasodilation during brief coronary artery occlusion or the early phase of reactive hyperemia, but that it does contribute to vasodilation during the late phase of reactive hyperemia.

In extracorporeally perfused dog hearts Aversano et al. (9) observed that glibenclamide in doses of 0.8 and 3.7 μmol/min caused 15% and 30% decreases in maximal coronary conductance, respectively, and caused 40% and 60% decreases in duration of the reactive hyperemia which followed a 30-s coronary occlusion. Total excess blood flow during reactive hyperemia was decreased by 60 and 70%, respectively. In open-chest dogs Imamura et al. (24) reported that intracoronary infusion of glibenclamide in a dose of 50 μg/kg per min decreased total reactive hyperemia excess flow by as much as 80%. In awake dogs the same dose of glibenclamide decreased excess blood flow during reactive hyperemia ~ 50% (8, and present study). Using the stroboscopic epiillumination technique to visualize the subepicardial coronary microvasculature in open-chest dogs, Kanatsuka et al. (26) showed that glibenclamide (200 μg/kg, intracoronary) inhibited dilation of coronary arterioles (< 100 μm) that occurred in response to a 20–30-s coronary artery occlusion, and markedly shortened the duration of vasodilation that occurred during the reactive hyperemia. These studies demonstrate that coronary arteriolar K^+_{ATP} channels contribute importantly to early and late post-ischemic reactive hyperemic responses in both perfused and intact hearts.

In the present study, the addition of adenosine receptor blockade did not affect the reactive hyperemia response to a 5-s coronary artery occlusion, compared to K^+_{ATP} channel blockade alone. In contrast, the addition of adenosine blockade did attenuate the peak coronary vasodilation after 10- and 20-s occlusions, suggesting that with longer occlusion durations, endogenous adenosine becomes progressively more important as an alternate vasodilator pathway when K^+_{ATP} channels are blocked.

Mechanism of interaction between adenosine and K^+_{ATP} channels. In the present study, the increases in CBF produced by intracoronary infusions of nitroprusside, adenosine, and pinacidil were measured to determine the selectivity and magnitude of K^+_{ATP} channel and adenosine receptor blockade produced by glibenclamide and 8-phenyltheophylline. The increase in CBF produced by nitroprusside was not altered by either K^+_{ATP} channel or adenosine receptor blockade, which is in agreement with earlier observations that nitroprusside causes vasodilation via an endothelium-independent nitric oxide/cGMP pathway (27). The coronary vasodilation produced by the K^+_{ATP} channel opener pinacidil was inhibited by glibenclamide but was not affected by adenosine receptor blockade with 8-phenyltheophylline. In contrast, the hyperemia produced by adenosine was attenuated by both 8-phenyltheophylline and by K^+_{ATP} channel blockade with glibenclamide. The latter observation is in agreement with previous reports that adenosine produces coronary vasodilation via activation of K^+_{ATP} channels (9, 28, 29). It is paradoxical that although glibenclamide inhibited the dilation caused by intracoronary infusions of adenosine, endogenous adenosine appeared to have a vasodilator effect during active and reactive hyperemia in the presence of gliben-

clamide. Inhibition of adenosine-induced vasodilation by glibenclamide has been reported to be competitive, i.e., the maximum response elicited by adenosine is not decreased by glibenclamide (29). Consequently, it is possible that in the presence of K^+_{ATP} channel blockade, adenosine accumulated in sufficient concentrations during exercise, to compete with glibenclamide and cause opening of K^+_{ATP} channels. This would imply that opening of K^+_{ATP} channels is mandatory for the increase in CBF produced by exercise. It has been proposed that under conditions of normal coronary arterial inflow, an increase in myocardial metabolic demands causes opening of K^+_{ATP} channels via intracellular signals (30). Although regulation of these channels by intracellular signals in vascular smooth muscle is not completely understood, a decrease in ATP or an increase in ADP levels near the sarcolemma, or phosphorylation of an internal regulatory site are thought to activate K^+_{ATP} channels (30). Adenosine receptors, which can activate K^+_{ATP} channels via a G-protein (30), do not appear to play a role in the channel activation process under normal arterial inflow conditions, since blockade of adenosine receptors does not interfere with the exercise-induced increase in CBF (1). However, when intracellular mechanisms to open K^+_{ATP} channels are inhibited by glibenclamide, a compensatory increase in interstitial adenosine concentration occurs which can result in adenosine receptor-mediated activation of the K^+_{ATP} channel. It is likely that when the adenosine concentrations are relatively low, e.g., under resting conditions, the K^+_{ATP} channel blocking (vasoconstrictor) effects of glibenclamide predominate over the K^+_{ATP} channel activating (vasodilator) actions of adenosine. However, when exercise in the presence of glibenclamide causes further deterioration of the myocardial oxygen supply-demand balance, adenosine concentrations are likely to increase to levels that can effectively compete with glibenclamide, resulting in activation of the channels and coronary vasodilation (14, 15). The reactive hyperemia experiments also support this hypothesis. Thus, while the addition of adenosine receptor blockade had no effect on the reactive hyperemia response to a 5-s coronary artery occlusion, reactive hyperemias after 10- and 20-s occlusions were attenuated compared to K^+_{ATP} channel blockade alone. It is likely that interstitial adenosine concentrations increase progressively with longer durations of coronary artery occlusion to compete with the effects of glibenclamide.

It is possible that adenosine opposed the vasoconstriction caused by glibenclamide in part via a different pathway not involving activation of K^+_{ATP} channels. Adenosine can cause vasodilation through adenylyl cyclase activation (31, 32). In support of this hypothesis, in the present study, the inhibition by glibenclamide of the vasodilation produced by adenosine was slightly less than that caused by the K^+_{ATP} channel activator pinacidil, suggesting that adenosine could have mediated part of its effect through another vasodilator pathway, likely adenylyl cyclase activation (31, 32).

In conclusion, blockade of K^+_{ATP} channels decreased CBF at rest but did not prevent the exercise-induced increase in blood flow. The mechanism for increased CBF in response to exercise after glibenclamide involves increased endogenous adenosine production, since the addition of adenosine receptor blockade inhibited the exercise-induced coronary vasodilation. Thus, while endogenous adenosine is not mandatory for the increase in CBF produced by exercise under normal conditions, it mediated the exercise-induced coronary vasodilation in response to exercise when K^+_{ATP} channels were blocked. These findings are compatible with the hypothesis that K^+_{ATP} channels do mediate coronary vasodilation in response to exercise. However, when intracellular activation of K^+_{ATP} channels is inhibited, adenosine provides an alternate pathway to mediate the coronary vasodilation in response to increases in myocardial oxygen demand produced by exercise.

Acknowledgments

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