Role of K^+_{ATP} Channels and Adenosine in the Regulation of Coronary Blood Flow during Exercise with Normal and Restricted Coronary Blood Flow

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Abstract

Regulation of coronary vasomotor tone during exercise is incompletely understood. We investigated the contributions of K^+_{ATP} channels and adenosine to the coronary vasodilation that occurs during exercise in the normal heart and in the presence of a coronary artery stenosis. Dogs that were chronically instrumented with a Doppler flow probe, hydraulic occluder, and indwelling catheter on the left anterior descending coronary artery were exercised on a treadmill to produce heart rates of ~ 200 beats/min. By graded inflation of the occluder to produce a wide range of coronary stenosis severities, we determined the coronary pressure–flow relation. K^+_{ATP} channel blockade with intracoronary glibenclamide (10–50 μg/kg per min) decreased coronary blood flow during exercise at coronary pressures within and below the autoregulatory range, indicating that coronary K^+_{ATP} channel activation is critical for producing coronary vasodilation with either normal arterial inflow or when flow is restricted by a coronary artery stenosis. Adenosine receptor blockade with intravenous 8-phenyltheophylline (5 mg/kg) had no effect on coronary flow at pressures within the autoregulatory range but decreased flow at pressures < 55 mmHg. In contrast, in the presence of K^+_{ATP} channel blockade, the addition of adenosine receptor blockade further decreased coronary flow even at coronary pressures in the autoregulatory range, indicating increased importance of the vasodilator influence of endogenous adenosine during exercise when K^+_{ATP} channels are blocked. Intracoronary adenosine (50 μg/kg per min) increased coronary flow at perfusion pressures both within and below the autoregulatory range. In contrast, selective K^+_{ATP} channel activation with intracoronary pinacidil (0.2–5.0 μg/kg per min) increased flow at normal but not at lower coronary pressures (< 55 mmHg). This finding demonstrates that not all K^+_{ATP} channels are activated during exercise at pressures in the autoregulatory range, but that most K^+_{ATP} channels are recruited as pressures approach the lower end of the autoregulatory plateau. Thus, K^+_{ATP} channels and endogenous adenosine play a synergistic role in maintaining vasodilation during exercise in normal hearts and distal to a coronary artery stenosis that results in myocardial hypoperfusion during exercise. (*J. Clin. Invest.* 1996. 97:996–1009.)
Key words: coronary blood flow • myocardial ischemia • myocardial oxygen consumption • regional myocardial systolic wall thickening

Introduction

In the normal heart, coronary blood flow is tightly coupled to metabolic demands to maintain a consistently high level of oxygen extraction by the myocardium. This close coupling, which is especially apparent during exercise, has been suggested to depend on messengers released from the myocardium or vascular endothelium. However, specific blockers of established endogenous vasodilators such as adenosine (1), prostacyclin (2), and nitric oxide (3) have not been found to impair coronary blood flow during exercise, indicating that these vasodilators are not mandatory for regulation of coronary vasomotor tone during exercise with normal coronary arterial inflow. In contrast, these vasodilator mechanisms can contribute significantly to regulation of coronary vasomotor tone during exercise in the presence of myocardial hypoperfusion. Thus, blockade of adenosine (4, 5) or nitric oxide (6) decreased coronary blood flow 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 contributes to regulation of coronary vasomotor tone (7). Thus, blockade of vascular smooth muscle K^+_{ATP} channels decreased coronary blood flow both under basal conditions (8–11) and during exercise with normal arterial inflow (11), and decreased coronary reactive hyperemia in response to a brief ischemic stimulus (8, 11). Studies in anesthetized open-chest dogs have suggested that the number of activated K^+_{ATP} channels increases progressively in response to restriction of coronary blood flow produced by a coronary artery stenosis (12, 13). However, no study has compared the role of K^+_{ATP} channels in regulation of coronary vasomotor tone during exercise in the presence of myocardial hypoperfusion with exercise during normal arterial inflow in the same animals.

The coronary pressure–flow relation describes the blood flow response over a range of perfusion pressures within and below the autoregulatory range, allowing a comprehensive assessment of regulation of coronary vasomotor tone. The behavior of coronary blood flow over a range of perfusion pressures is relevant to the clinical situation in which an arterial stenosis can result in decreased perfusion pressure. To determine the contribution of K^+_{ATP} channels to the regulation of coronary blood flow under conditions of normal and restricted arterial inflow, we studied the effects of the selective K^+_{ATP}

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channel antagonist glibenclamide and the selective agonist pinacidil on the coronary pressure–flow relation in awake exercising dogs. Since it has been demonstrated that, in the canine coronary circulation, adenosine produces coronary vasodilation in part via activation of K^+_{ATP} channels (8), we also assessed the interaction between endogenous adenosine and K^+_{ATP} channels during exercise.

Methods

Studies were performed in 25 adult mongrel dogs weighing 20–27 kg and trained to run on a motor-driven treadmill. All experiments were 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, i.m.), dogs were anesthetized with sodium pentobarbital (30–35 mg/kg, i.v.), 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 o.d.) filled with heparinized saline was inserted into the left internal thoracic artery and advanced into the ascending aorta. Similar catheters were introduced into the right 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 through the area of the apex. Approximately 1.5 cm of the proximal left anterior descending coronary artery (LAD)[1] was dissected free, and a Doppler flow probe (Craig Hartley Methodist Hospital, Houston, TX) was positioned around the artery. Immediately distal to the flow probe, a hydraulic occluder (3.0 mm o.d.) was placed around the vessel. A silicone catheter (0.3 mm, i.d.) bonded to a larger silicone catheter (1.6 mm i.d.) 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 myocardial region perfused by the LAD and the control region perfused by the left circumflex coronary artery (LCX), respectively. The pericardium was then loosely closed, and the catheters and electrical leads were tunneled subcutaneously to exit at the base of the neck. The chest was closed in layers, and the pneumothorax was evacuated. Catheters were flushed daily with heparinized saline.

Hemodynamic measurements

Studies were performed 2–3 wk after surgery with animals exercising on a motor-driven treadmill. Recordings of phasic and mean aortic pressure were obtained with pressure transducers (P23XL; Gould Inc., Cleveland, OH) positioned at mid-chest level. Left ventricular pressure was measured with the micromanometer calibrated with the fluid-filled left ventricular catheter. Left ventricular dP/dt was obtained via electrical differentiation of the left ventricular pressure signal. Coronary Doppler shift was measured with a Doppler flowmeter system (Craig Hartley). Data were recorded on an eight-channel direct writing oscillograph (Coulbourn Instruments, Inc., Lehigh Valley, 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. End-diastolic wall thickness (EDT) was measured at the onset of positive LVdP/dt and end-systolic wall thickness (EST) was measured 20 ms before peak negative LVdP/dt. Percent myocardial systolic wall thickening (SWT) was computed as follows:

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

Experimental protocols

To test the magnitude and selectivity of the agonists and antagonists used in the present study, dose response studies were performed in a total of seven dogs standing quietly in a sling.

Magnitude and selectivity of K^+ATP channel blockade produced by glibenclamide. In six resting dogs, the magnitude and selectivity of glibenclamide as a K^+_{ATP} channel blocker was assessed. For this purpose we measured the increases in coronary blood flow produced 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 of five dogs. After washout of pinacidil, an infusion of glibenclamide was started into the coronary artery in a dose of 10 μg/kg per min at a rate of 0.3 ml/min. While the glibenclamide infusion was continued, the pinacidil infusions were repeated (0.5, 1, and 2.5 μg/kg per min), and coronary blood flow measurements were obtained. The infusion of glibenclamide was then increased to 50 μg/kg per min at a rate of 1.5 ml/min, and the pinacidil infusions were repeated (0.5, 1, 2.5, and 5 μg/kg per min). On separate days, we studied the effects of glibenclamide on the increases in coronary blood flow produced by nitroprusside (0.6, 1.5, and 3.0 μg/kg per min; $n = 5$) and adenosine (1, 2.5, 5, 10, 25, and 50 μg/kg per min; $n = 6$).

Glibenclamide had no effect on the increase in coronary flow caused by nitroprusside but markedly decreased the coronary vasodilation by pinacidil (Fig. 1). Thus, glibenclamide in a dose of 50 μg/kg per min caused 85±5% inhibition of the increase in coronary flow caused by 2.5 μg/kg per min pinacidil. The coronary blood flow responses to adenosine were also markedly inhibited, although slightly less than the pinacidil-induced hyperemia as the increase in flow produced by 25 μg/kg per min adenosine was decreased by 75±5%. Conversely, a 10-fold higher dose of adenosine and an ~ 20-fold higher dose of pinacidil were required to elicit a 10-ml/min increase in coronary blood flow in the presence of glibenclamide compared with control conditions (Fig. 1).

Magnitude and selectivity of adenosine receptor blockade produced by 8-phenyltheophylline. In four resting dogs (two of which were also studied in the above-described glibenclamide protocol), the magnitude and selectivity of adenosine receptor blockade produced by 8-phenyltheophylline was determined. For this purpose, we measured the increases in coronary blood flow 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), administered in random order. After completion of these measurements, 8-phenyltheophylline was administered into the right atrial catheter in a dose of 5 mg/kg over a period of 5 min. 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. The increases in coronary blood flow produced by intracoronary infusions of pinacidil and nitroprusside were not altered by 8-phenyltheophylline, but those produced by adenosine were markedly attenuated (Fig. 2).

Exercise protocols

Exercise studies were performed in a total of 20 dogs (two of which were also studied in the dose response studies). In several of the dogs, more than one protocol was performed; in these animals exercise protocols were executed in random order.

Control group. In 11 dogs, the reproducibility of the coronary pressure–flow relation and coronary pressure–systolic wall thickening (pressure–function) relations during treadmill exercise were studied. With the dogs standing on the treadmill, resting measurements of sys-

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

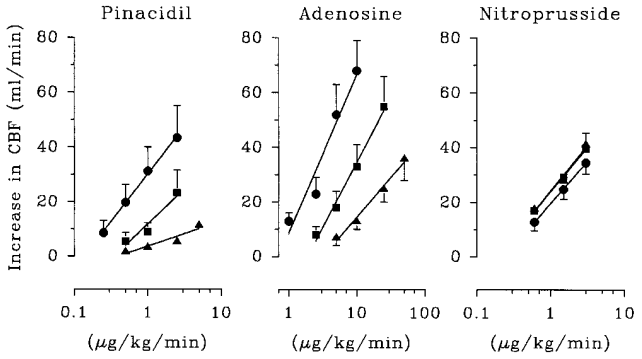

Figure 1. Effects of glibenclamide on increases in coronary blood flow from baseline produced by intracoronary infusions of pinacidil ($n = 5$), adenosine ($n = 6$), and nitroprusside ($n = 5$) in awake resting dogs. Shown are the blood flow responses during control (*circles*); glibenclamide, 10 μg/kg per min, intracoronary (*squares*); and glibenclamide, 50 μg/kg per min, intracoronary (*triangles*). Data are mean±SEM.

temic and coronary hemodynamic variables and regional wall thickness were obtained. Dogs underwent a 5-min period of warm-up exercise during which the speed and grade of the treadmill were gradually increased until a heart rate of ~ 200 beats/min was achieved. This usually required treadmill exercise at 6.4 km/h and 5% incline. Animals were subsequently allowed to rest on the treadmill for 10–15 min, and exercise was then restarted at the predetermined level. After 2 min of exercise, when hemodynamic variables had reached a steady state, the occluder was inflated with saline using a micrometer-driven syringe to produce progressively increasing severity of coronary artery stenosis until the LAD was totally occluded. At each level of stenosis, a minimum of 15 s was allowed (during which hemodynamic data reached a new stable level) before systemic and coronary hemodynamics were measured. The occluder was then deflated, exercise was discontinued, and the animals were allowed to rest. A total of 10–20 coronary pressure–flow and pressure–function data points were obtained under conditions varying from no stenosis to total coronary artery occlusion. In total, animals underwent three exercise periods (each lasting 8–14 min) separated by 90 min of rest. Five animals received intracoronary infusions of saline during the second (0.3 ml/min) and third (1.5 ml/min) run, whereas six dogs did not receive an infusion of saline. The data from these two groups were not different and have therefore been analyzed together.

The coronary pressure–flow relation and the coronary pressure–function relation during exercise were characterized by a plateau at coronary pressures > 80 mmHg (Fig. 3). As coronary pressure decreased below 80 mmHg, coronary flow and systolic wall thickening decreased progressively in parallel with coronary pressure, reaching zero flow at a coronary pressure of 36±2 mmHg; systolic wall thickening was −2±3% during total coronary artery occlusion. The second and third exercise period produced pressure–flow and pressure–function relations that were identical to those measured during the first exercise period, demonstrating excellent reproducibility of these relations during three consecutive exercise periods (Fig. 3).

Glibenclamide group. The effects of K^+_{ATP} channel blockade on the coronary pressure–flow and pressure–function relations during exercise were studied in 11 dogs (of which 8 dogs were also studied in the control group). With dogs standing on the treadmill, resting measurements of systemic and coronary hemodynamic variables and regional wall thickness were obtained. Then animals were exercised, and the coronary pressure–flow and pressure–function relations were determined under control conditions, as described above. After 90 min of rest, an infusion of glibenclamide was started into the coronary artery in a dose of 10 μg/kg per min, delivered at a rate of 0.3 ml/min. 5 min later, resting measurements were obtained, and the exercise protocol was repeated. After another 90 min of rest, the exercise protocol was repeated in the presence of glibenclamide, infused in a dose of 50 μg/kg per min (1.5 ml/min).

8-Phenyltheophylline group. The effects of adenosine receptor blockade on the coronary pressure–flow and pressure–function relations during exercise were studied in seven dogs (of which two animals were also studied in the control group). With dogs standing on the treadmill, resting measurements of systemic and coronary hemodynamic variables and regional wall thickness were obtained. Animals were then exercised, and the coronary pressure–flow and pressure–function relations were determined under control conditions, as described above. After 90 min of rest, 8-phenyltheophylline was ad-

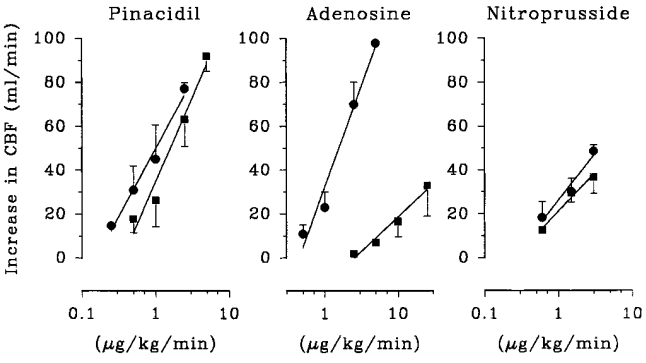

Figure 2. Effects of 8-phenyltheophylline (5 mg/kg, intravenous) on increases in coronary blood flow from baseline produced by intracoronary infusions of pinacidil ($n = 4$), adenosine ($n = 4$), and nitroprusside ($n = 4$) in awake resting dogs. Shown are the blood flow responses during control (*circles*) and 8-phenyltheophylline, 5 mg/kg, intravenous (*squares*). Data are mean±SEM.

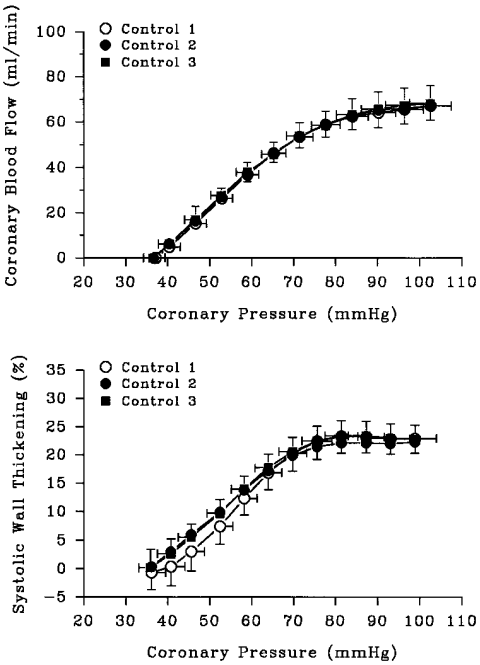

Figure 3. Coronary pressure–flow and –function relations in dogs undergoing treadmill exercise. The relation between LAD coronary pressure and blood flow is displayed in the upper panel ($n = 11$); the relation between LAD coronary pressure and systolic thickening in the anterior left ventricular wall is displayed in the lower panel ($n = 7$). Shown are the relations during three consecutive control periods, control 1 (*open circles*), control 2 (*closed circles*), and control 3 (*squares*). Data are mean±SEM.

ministered intravenously in a dose of 5 mg/kg infused over 5 min, and 10 min later the exercise protocol was repeated.

Glibenclamide and 8-phenyltheophylline group. The effects of K^+_{ATP} channel blockade on the coronary pressure–flow and pressure–function relations during exercise were studied in the absence and presence of adenosine receptor blockade in six dogs (of which two animals were also studied in the control group and three in the 8-phenyltheophylline group). With the dogs standing on the treadmill, resting measurements of systemic and coronary hemodynamic variables and regional wall thickness were obtained. Animals were then exercised, and the coronary pressure–flow and pressure–function relations were determined under control conditions as described above. After 90 min of rest, an infusion of glibenclamide (50 μg/kg per min) was begun into the coronary artery catheter delivered at a rate of 1.5 ml/min. 5 min after beginning the infusion, resting measurements were obtained, and the exercise protocol was repeated. After completion of the exercise protocol, the glibenclamide infusion was discontinued, and the animals were allowed to rest for 90 min. 8-Phenyltheophylline was then administered intravenously in a dose of 5 mg/kg infused over 5 min. 10 min later, the intracoronary infusion of glibenclamide (50 μg/kg per min) was restarted, and 5 min later the exercise protocol was repeated.

Pinacidil group. The effects of K^+_{ATP} channel activation on the coronary pressure–flow and pressure–function relations during exercise were studied in 11 dogs (of which 6 dogs were also studied in the control group, 3 in the glibenclamide group, and 2 in the glibenclamide and 8-phenyltheophylline group). With dogs standing on the treadmill, resting measurements of systemic and coronary hemodynamic variables and regional wall thickness were obtained. The animals were then exercised, and the coronary pressure–flow and pressure–function relations determined under control conditions as previously described. After 90 min of rest, an infusion of pinacidil into the coronary artery was started in a dose of 0.2 μg/kg per min, delivered at a rate of 0.3 ml/min. 5 min later, resting measurements were obtained, and the exercise protocol was repeated. After another 90 min of rest, the exercise protocol was repeated in the presence of pinacidil infused in a dose of 1.0 μg/kg per min, delivered at a rate of 1.5 ml/min.

High-dose pinacidil group. The effects of a high dose of pinacidil on the coronary pressure–flow and pressure–function relations were studied in six dogs (of which two animals were studied in the control group, one in 8-phenyltheophylline group, and three in the pinacidil group). Ultrasonic crystals were implanted in five of these animals, but reliable tracking of the signal in the LAD region could be obtained in only three of these animals. Animals were exercised, and the coronary pressure–flow relation was determined under control conditions as described above. After 90 min of rest, the exercise protocol was repeated during infusion of pinacidil into the coronary artery in a dose of 5 μg/kg per min, delivered at a rate of 0.6 ml/min.

High-dose adenosine group. The effects of maximal vasodilation with adenosine on the coronary pressure–flow and pressure–function relations were studied in six dogs (of which two animals were studied in the control group, two in the pinacidil group, and two in the high-dose pinacidil group). Ultrasonic crystals were implanted in four of these animals, but reliable tracking of the signal in the LAD region could be obtained in only two of these animals. Animals were exercised, and the coronary pressure–flow relation was determined under control conditions as described above. After 90 min of rest, the exercise protocol was repeated during infusion of adenosine into the coronary artery in a dose of 50 μg/kg per min, delivered at a rate of 0.6 ml/min. Maximal vasodilation was demonstrated by the absence of reactive hyperemia in response to a 15-s coronary artery occlusion and by the lack of a further increase in coronary flow in response to an increase in adenosine infusion rate.

Data analysis

Heart rate, left ventricular and aortic and coronary pressures, coronary Doppler shift, and regional wall thickness of the LAD-perfused region and the LCX-perfused control region were measured from the strip chart recordings. Coronary blood flow was computed from the Doppler shift using the equation $Q = 2.5 \cdot \Delta f \cdot d^2$, where Q is the coronary blood flow (ml/min), Δf is the Doppler shift (KHz), and d is the internal diameter of the coronary artery (mm) within the flow probe (14). 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 sound beam emitted ($f_0 = 10$ MHz), the cosine of the angle at which the sound beam is emitted (45°), and unit conversion factors: $(C \cdot \pi/4 \cdot 3)/(2f_0 \cdot \cos 45°)$. Since in the chronically instrumented animals the flow probe adheres 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 $\sim 20\%$ of the external diameter of the coronary artery. In this way, errors in the computation of the coronary internal diameter would affect control and intervention conditions equally.

Optimal curve fitting of the coronary pressure–flow and pressure–function data was obtained with a fourth-order polynomial ($y = a + bx + cx^2 + dx^3 + ex^4$), and coronary flows were computed at several coronary pressures within the range of coronary pressures measured during each protocol.

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

Drugs

Pinacidil was dissolved in deionized water, pH 6.0–6.5. Glibenclamide was dissolved in deionized water, pH 8.0–8.5. 8-Phenyltheophylline was dissolved in dimethyl sulfoxide and deionized water, pH 10.0–11.0. Adenosine was dissolved in physiologic saline. Sodium nitroprusside was dissolved in 5% dextrose. All drugs were infused directly into the coronary artery catheter, with the exception of 8-phenyltheophylline. Because of the high pH (10–11) of the solution, 8-phenyltheophylline was administered intravenously to avoid interference with coronary vasomotor tone regulation and to prevent precipitation due to mixing with other drug solutions during infusion via the single coronary artery catheter.

Results

Glibenclamide group

Systemic hemodynamics. Exercise increased heart rate from 122±4 at rest to 193±6 beats/min ($P < 0.01$), mean arterial pressure from 95±3 to 106±3 mmHg ($P < 0.01$), left ventricular systolic pressure from 115±5 to 134±5 mmHg ($P < 0.01$), and $LVdP/dt_{max}$ from 2,400±170 to 4,140±280 mmHg/s ($P < 0.01$), but did not significantly alter left ventricular end-diastolic pressure (Table I). While exercise was continued, progressive coronary artery occlusion caused a marked increase in left ventricular end-diastolic pressure from 9±2 mmHg during exercise with normal coronary arterial inflow to 16±2 mmHg during exercise with total coronary artery occlusion ($P < 0.05$). This was accompanied by a slight decrease in left ventricular systolic pressure to 122±5 mmHg ($P < 0.05$) and a small increase in heart rate to 217±5 beats/min ($P < 0.05$).

Glibenclamide produced an increase in left ventricular end-diastolic pressure and heart rate under resting conditions, while producing small decreases in left ventricular systolic

Table I. Hemodynamic and Contractile Function Data during K^+_{ATP} Channel Blockade

	Heart rate			Mean aortic pressure			LV systolic pressure			End-diastolic pressure			$LVdP/dt_{max}$		
	Con	G10	G50	Con	G10	G50	Con	G10	G50	Con	G10	G50	Con	G10	G50
	beats/min			*mmHg*			*mmHg*			*mmHg*			*mmHg/s*		
Rest	122±4	126±7	144±6*	95±3	98±5	97±5	115±5	119±7	111±5	6±1	8±1	9±1*	2,400±170	2,410±250	2,240±170
Exercise	193±6‡	193±7‡	190±5‡	106±3‡	104±4‡	104±4‡	134±5‡	128±5*‡	126±6*‡	9±2	10±1‡	13±2‡	4,140±280‡	3,650±280*‡	3,370±210*‡
Exercise + occlusion	217±6‡§	214±7‡§	207±6*‡§	102±4‡	100±4	101±3	128±5‡§	122±5§	119±4*‡	16±2‡§	16±2‡§	17±2‡§	4,030±280‡	3,660±320*‡	3,670±210‡§
	Coronary pressure			Coronary blood flow			EDT			EST			SWT		
	Con	G10	G50	Con	G10	G50	Con	G10	G50	Con	G10	G50	Con	G10	G50
	mmHg			*ml/min*			*mm*			*mm*			%		
Rest	95±4	97±4	94±5	47±4	45±4	40±4*	8.6±0.7	8.6±0.7	8.8±0.9	10.2±0.7	10.0±0.8	9.7±1.0	19±2	16±3	10±2*
Exercise	104±3‡	104±4‡	103±4	71±6‡	63±5*‡	55±6*‡	8.8±0.8	8.9±0.8	8.9±1.0	10.9±0.9‡	10.5±1.0	10.0±1.1*	23±3	18±4*	12±3*
Exercise + occlusion	31±3‡§	33±3*‡§	37±3*‡§	0	0	0	8.4±0.9§	8.5±0.8§	8.6±0.1‡§	8.3±0.6‡§	8.3±0.7‡§	8.4±0.7‡§	0±3‡§	−1±3‡§	−2±3‡§

Values are mean±SEM. $n = 11$; $n = 7$ for left ventricular end-diastolic (EDT) and end-syotolic (EST) wall thickness and systolic wall thickening (SWT) in the LAD region.
*$P < 0.05$ vs corresponding control measurements, ‡$P < 0.05$ vs rest, §$P < 0.05$ exercise vs exercise + occlusion, *Con* control; *G10*, glibenclamide, 10 μg/kg per min i.c.; *G50*, glibenclamide, 50 μg/kg per min i.c.

Table II. Hemodynamic Data during Adenosine Receptor Blockade

	Heart rate		Mean aortic pressure		LV systolic pressure		End-diastolic pressure		$LVdP/dt_{max}$		Coronary pressure		Coronary blood flow	
	Con	8PT	Con	8PT	Con	8PT	Con	8PT	Con	8PT	Con	8PT	Con	8PT
	beats/min		*mmHg*		*mmHg*		*mmHg*		*mmHg/s*		*mm Hg*		*Ml/min*	
Rest	125±7	137±7	99±4	105±5	123±4	130±4	8±1	9±2	3,120±230	3,270±310	94±3	94±6	49±10	50±9
Exercise	203±5*	204±7*	112±5*	1,135±5*	144±5*	142±4*	10±2	11±2	5,480±360*	5,360±440*	99±6	96±6	78±13*	76±12*
Exercise + occlusion	209±6*	214±6*	110±4*	108±6*‡	138±4*‡	136±6*	19±4*‡	19±3*‡	4,960±270*‡	5,060±420*	33±4*‡	36±5*‡§	0	0

Values are mean±SEM. $n = 7$. *$P < 0.05$ vs rest, ‡$P < 0.05$ exercise vs exercise + occlusion, §$P < 0.05$ vs. corresponding control measurements. *Con*, control; *8PT*, 8-phenyltheophylline, 5 mg/kg i.v.

pressure and dP/dt_{max} during exercise (Table I). During exercise in the presence of a total coronary artery occlusion, heart rate, left ventricular systolic pressure, and dP/dt_{max} were slightly lower in the presence of glibenclamide.

Coronary hemodynamics. Control exercise increased coronary pressure from 95±4 mmHg at rest to 104±3 mmHg ($P < 0.05$), and coronary blood flow from 47±4 ml/min at rest to 71±6 ml/min ($P < 0.01$) (Table I). Progressive coronary artery occlusion decreased coronary pressure to 31±3 mmHg during exercise in the presence of total coronary artery occlusion. Glibenclamide had no effect on coronary artery pressure at rest or during exercise. In contrast, glibenclamide 50 μg/kg per min decreased coronary blood flow at rest from 47±4 ml/min to 40±4 ml/min ($P < 0.05$) and from 71±6 to 55±6 ml/min during exercise ($P < 0.01$) (Table I). Glibenclamide caused a dose-dependent increase in the coronary pressure at zero flow during exercise to 37±3 mmHg ($P < 0.05$) (Table II). Since the extravascular determinants of pressure at zero flow (left ventricular end-diastolic pressure and heart rate) were not altered by glibenclamide, the increase in pressure at zero flow must have been due to an increase in vasomotor tone. Glibenclamide decreased coronary blood flow during exercise at all coronary pressures, so that absolute flow reductions were similar at all pressures (Fig. 4). Thus, the decrease in coronary blood flow caused by glibenclamide was independent of coronary pressure.

Myocardial function. Control exercise had no effect on end-diastolic wall thickness but increased end-systolic wall thickening in both the myocardial region perfused by the LAD and the control region perfused by the LCX. As a result, systolic wall thickening increased from 19±2% at rest to 23±3% during exercise in the LAD region (Table I), and from 17±2% to 23±2% in the circumflex control region (not shown). In the LAD region, glibenclamide had no effect on end-diastolic wall thickness but decreased end-systolic wall thickening. Consequently, glibenclamide decreased systolic wall thickening in the LAD region to 10±2% at rest and to 12±3% during exercise (both $P < 0.05$), with no effect on wall thickening in the control region. The coronary pressure–function relation during exercise paralleled the changes observed in the pressure–flow relation (Fig. 4). Systolic wall thickening in the control region was maintained during progressive occlusion of the LAD (not shown).

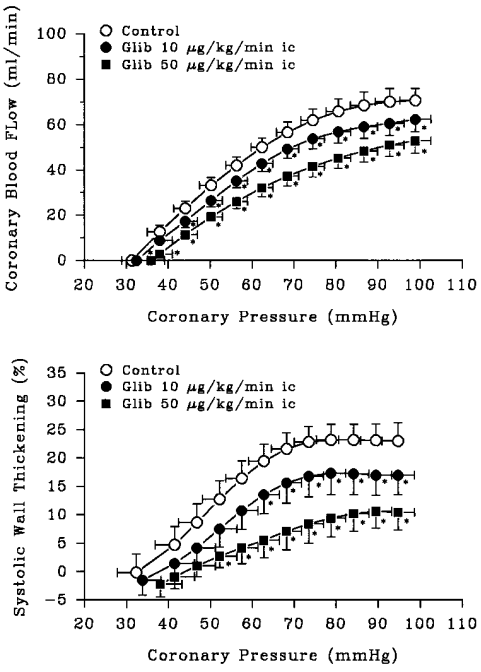

Figure 4. Coronary pressure–flow and –function relations in dogs undergoing treadmill exercise. The relation between LAD coronary pressure and blood flow is displayed in the upper panel ($n = 11$); the relation between LAD coronary pressure and systolic thickening in the anterior left ventricular wall is displayed in the lower panel ($n = 6$). Shown are the relations during control (*open circles*); glibenclamide, 10 μg/kg per min, intracoronary (*closed circles*); and glibenclamide, 50 μg/kg per min, intracoronary (*squares*). Data are mean±SEM. *$P < 0.05$ versus corresponding control measurement.

8-Phenyltheophylline group

Systemic hemodynamics. 8-Phenyltheophylline (5 mg/kg, i.v.) had no effect on any systemic hemodynamic variable at rest or during exercise (Table II).

Coronary hemodynamics. 8-Phenyltheophylline had no effect on either coronary pressure or coronary blood flow at rest or during exercise with normal arterial inflow. In contrast, 8-phenyltheophylline caused a small but significant decrease in coronary blood flow at coronary pressures of 50 mmHg and below (Fig. 5), and increased the Pzf from 33±4 to 36±5 mmHg ($P < 0.05$) (Table II).

Myocardial function. 8-Phenyltheophylline had no effect on systolic wall thickening at rest or during exercise with normal arterial inflow. The coronary pressure–function relation during exercise paralleled the changes observed in the pressure–flow relation (not shown). Systolic wall thickening in the control region was maintained during progressive occlusion of the LAD.

8-Phenyltheophylline and glibenclamide group

Systemic hemodynamics. The addition of 8-phenyltheophylline (5 mg/kg i.v.) to glibenclamide (50 μg/kg per min, i.c.) did not produce further changes in hemodynamics compared with glibenclamide alone, except for a slightly lower heart rate during exercise in the presence of a total coronary artery occlusion (Table III).

Coronary hemodynamics. The addition of 8-phenyltheophylline to glibenclamide further decreased coronary blood flow compared with glibenclamide alone, and this reached lev-

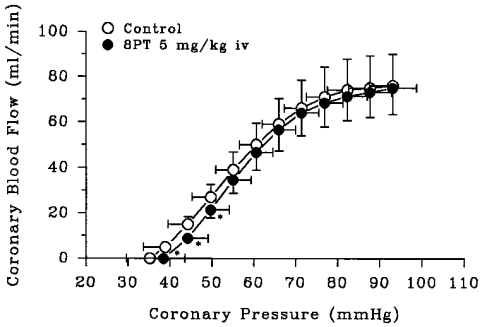

Figure 5. Coronary pressure–flow relation in dogs undergoing treadmill exercise. Shown are the relations during control (*open circles*) and 8-phenyltheophylline, 5 mg/kg, intravenous (*closed circles*). Data are mean±SEM, $n = 7$. *$P < 0.05$ versus corresponding control measurement.

Table III. Hemodynamic and Contractile Function Data during Combined K^+_{ATP} Channel Blockade and Adenosine Receptor Blockade

	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
	beats/min			*mmHg*			*mmHg*			*mmHg*			*mmHg/s*		
Rest	117±7	131±7	144±7	96±4	101±4	102±8	121±5	120±5	118±8	9±1	11±1	10±1	2,650±280	2,460±160	2,540±150
Exercise	194±4*	201±7*	190±9*	110±5*	107±4	105±8	141±4*	132±4*‡	126±8*	12±1	13±2	15±2*	4,670±480*	4,170±490*‡	3,890±300*‡
Exercise + occlusion	196±4*	199±6*	184±10*‖	107±5*	108±5	108±6	130±4§	128±6*	125±7	21±2*§	20±3*§	20±2*§	4,330±380*	3,990±310*	3,770±260*‡
	Coronary pressure			Coronary blood flow			End-diastolic thickness			End-systolic thickness			Systolic thickening		
	Con	G	8pt+G	Con	G	8PT+G	Con	G	8PT+G	Con	G	8PT+G	Con	G	8PT+G
	mmHg			*mmHg*											
Rest	95±4	99±4	100±9	43±3	33±5	26±3	7.3±0.2	7.2±0.3	7.1±0.2	8.5±0.2	7.6±0.3‡	7.4±0.4‡	15±3	6±2‡	5±3‡
Exercise	104±4*	103±5	104±7	68±6*	47±8*‡	33±5*‡‖	7.3±0.2	7.1±0.3	7.0±0.3	8.6±0.5	7.7±0.3‡	7.3±0.3‡‖	19±4	11±3*‡	5±2‡‖
Exercise + occlusion	35±4*§	46±7*§§	55±8*§§¶	0	0	0	6.6±0.3*§	6.8±0.4§	6.8±0.2§	6.8±0.4*§	6.9±0.4	6.9±0.4	2±1*§	1±1§	1±2

Values are mean±SEM. $n = 6$; $n = 5$ for left ventricular end-diastolic (EDT) and end-systolic (EST) wall thickness and systolic wall thickening (SWT) in the LAD region. $^*P < 0.05$ vs rest, $^‡P < 0.05$ vs corresponding control measurement, $^§P < 0.05$ exercise vs exercise + occlusion, $^‖P < 0.05$, $^¶P < 0.06$ vs corresponding glibenclamide measurement. *Con*, control; *G*, glibenclamide, 50 μg/kg per min i.c.; 8PT+G = 8-phenyltheophylline, 5 mg/kg i.v. + glibenclamide, 50 μg/kg per min i.c.

Figure 6. Coronary pressure–flow and –function relations in dogs undergoing treadmill exercise. The relation between LAD coronary pressure and blood flow is displayed in the upper panel ($n = 6$); the relation between LAD coronary pressure and systolic thickening in the anterior left ventricular wall is displayed in the lower panel ($n = 6$). Shown are the relations during control (*open circles*); glibenclamide, 50 μg/kg per min, intracoronary (*closed circles*); and glibenclamide, 50 μg/kg per min, intracoronary, with 8-phenyltheophylline, 5 mg/kg, intravenous (*squares*). Data are mean±SEM, $n = 6$. $^*P < 0.05$ versus corresponding control measurement. $^†P < 0.05$ versus corresponding glibenclamide measurement.

els of significance during exercise (Table III). The addition of 8-phenyltheophylline decreased coronary blood flow at all coronary pressures during exercise (Fig. 6), so that the absolute flow reductions were similar at each pressure. Thus, the decrease in coronary flow produced by 8-phenyltheophylline in the presence of glibenclamide was independent of coronary pressure. Glibenclamide increased coronary zero flow pressure from 35±4 to 46±7 mmHg during exercise ($P < 0.05$); the addition of 8-phenyltheophylline further increased coronary pressure at zero flow during exercise to 55±8 mmHg ($P = 0.06$) (Table III).

Myocardial function. The addition of 8-phenyltheophylline had no effect on end-diastolic wall thickness but further decreased end-systolic wall thickening in the LAD region (Table III). Consequently, systolic wall thickening in the LAD region decreased from 11±3% during exercise in the presence of glibenclamide to 5±2% ($P < 0.05$), with no effect on wall thickening in the control region. The coronary pressure–function relation during exercise paralleled the changes observed in the pressure–flow relation (Fig. 6). Systolic wall thickening in the control region was maintained during progressive occlusion of the LAD (not shown).

Pinacidil group

Systemic hemodynamics. Pinacidil (0.2–1.0 μg/kg per min, i.c.) had no effect on any of the systemic hemodynamics either at

Table IV. Hemodynamic Data during K^+_{ATP} Channel Activation

	Heart rate			Mean aortic pressure			LV systolic pressure			End-diastolic pressure			$LVdP/dt_{max}$		
	Con	P 0.2	P 1.0	Con	P 0.2	P 1.0	Con	P 0.2	P 1.0	Con	P 0.2	P 1.0	Con	P 0.2	P 1.0
	beats/min			*mmHg*			*mmHg*			*mmHg*			*mmHg/s*		
Rest	115±3	117±6	116±5	100±3	101±4	102±3	122±4	119±4	123±4	6±1	8±2	8±1	2,560±160	2,490±190	2,610±200
Exercise	186±5*	185±5*	190±5*	110±4*	108±4*	110±4*	141±4*	136±3*	137±3*	10±2*	8±2	9±2	4,210±320*	4,140±320*	4,070±330*
Exercise + occlusion	240±4*‡	270±6*‡	209±6*‡	106±3	104±4	106±3	130±4	128±4‡	128±3*‡	16±4*‡	18±3*‡	17±3*‡	4,050±280*	4,050±350*	3,990±300*

	Coronary pressure			Coronary blood flow		
	Con	P 0.2	P 1.0	Con	P 0.2	P 1.0
	mmHg			*ml/min*		
Rest	99±3	98±3	100±3	41±2	51±3§	70±5§
Exercise	108±5*	105±5	104±5	63±3*	69±4*§	85±6*§
Exercise + occlusion	33±3*‡	32±3*‡	33±3*‡	0	0	0

Values are mean±SEM. $n = 11$; $*P < 0.05$ vs rest, $‡P < 0.05$ exercise vs exercise + occlusion, $§P < 0.05$ vs corresponding control measurements. *Con*, control; *P 0.2*, pinacidil, 0.2 μg/kg per min i.c.; *P 1.0*, pinacidil, 1.0 μg/kg per min i.c.

rest or during exercise with normal arterial inflow or coronary artery occlusion (Table IV).

Coronary hemodynamics. Pinacidil had no effect on coronary artery pressure at rest or during exercise with normal arterial inflow. Coronary blood flow increased from 41±2 ml/min under control conditions to 70±5 ml/min during pinacidil, 1.0 μg/kg per min, at rest ($P < 0.01$) and from 63±3 to 85±6 ml/min during exercise ($P < 0.01$) (Table IV). Progressive coronary artery occlusion decreased coronary pressure to 33±3 mmHg during control exercise in the presence of total coronary artery occlusion. Pinacidil did not alter the coronary pressure at zero flow during exercise (Table IV). Pinacidil increased coronary blood flow during exercise at perfusion pressures > 65 mmHg but had no effect on coronary flow at pressures < 65 mmHg (Fig. 7).

Myocardial function. Pinacidil had no effect on systolic wall thickening in either the LAD region or the control region at rest or during exercise (not shown). The coronary pressure–function relations in the LAD region during exercise was also not affected by pinacidil.

High-dose pinacidil group

Systemic hemodynamics. The high dose of pinacidil (5 μg/kg per min, i.c.) had no effect on any of the systemic hemodynamics either at rest or during exercise with normal arterial inflow or coronary artery occlusion (Table V).

Coronary hemodynamics. The high dose of pinacidil caused a mild decrease in coronary artery pressure which reached significance during exercise with normal arterial inflow. Coronary blood flow increased from 41±3 ml/min under control conditions to 137±13 ml/min during pinacidil, 5.0 μg/kg per min ($P < 0.05$), at rest, and from 67±5 to 132±10 ml/min during exercise ($P < 0.05$) (Table V). The high dose of pinacidil did not alter the coronary pressure at zero flow during exercise. The high dose of pinacidil increased coronary blood flow during exercise at coronary pressures > 55 mmHg but had no effect on coronary flow at pressures < 55 mmHg (Fig. 8).

Myocardial function. The high dose of pinacidil had no effect on systolic wall thickening in either the LAD region or the control region at rest or during exercise (not shown). The coronary pressure–function relation in the LAD region during exercise was also not affected by the high dose of pinacidil (not shown).

Figure 7. Coronary pressure–flow relation in dogs undergoing treadmill exercise. Shown are the relations during control (*open circles*); pinacidil, 0.2 μg/kg per min, intracoronary (*closed circles*); and pinacidil, 1.0 μg/kg per min, intracoronary (*squares*). Data are mean±SEM, $n = 11$. $*P < 0.05$ versus corresponding control measurement.

Table V. Hemodynamic Data during K^+_{ATP} Channel Activation

	Heart rate		Mean aortic pressure		LV systolic pressure		End-diastolic pressure		$LVdP/dt_{max}$		Coronary pressure		Coronary blood flow	
	Con	P5	Con	P5	Con	P5	Con	P5	Con	P5	Con	P5	Con	P5
	beats/min		*mmHg*		*mmHg*		*mmHg*		*mmHg/s*		*mmHg*		*ml/min*	
Rest	123±8	136±7	99±5	102±4	120±6	122±4	8±1	7±1	2,510±110	2,670±90	99±5	89±7	41±3	137±13*
Exercise	201±7‡	200±6‡	111±5‡	109±4‡	140±4‡	135±4‡	8±2	9±2	4,360±170‡	4,340±190‡	107±6	91±6*	67±5‡	132±10*
Exercise + occlusion	212±4‡	208±7‡	105±6‡§	107±4	133±6‡§	131±3	21±4‡§	23±1‡§	4,020±130‡	4,300±380‡	33±5‡§	34±4‡§	0	0

Values are mean±SEM. $n = 6$; * $P < 0.05$ vs corresponding control measurements, ‡ $P < 0.05$ vs rest, § $P < 0.05$ exercise vs exercise + occlusion. *Con*, control; *P5*, pinacidil, 5 μg/kg per min i.c.

Figure 8. Coronary pressure–flow relation in dogs undergoing treadmill exercise. Shown are the relations during control (*open circles*) and pinacidil, 5 μg/kg per min, intracoronary (*closed circles*). Data are mean±SEM, $n = 6$. * $P < 0.05$ versus corresponding control measurement.

High-dose adenosine group

Systemic hemodynamics. Adenosine (50 μg/kg per min, i.c.) had no effect on any of the systemic hemodynamics either at rest or during exercise with normal arterial inflow or coronary artery occlusion (Table VI).

Coronary hemodynamics. The high dose of adenosine caused a decrease in coronary artery pressure at rest and during exercise with normal arterial inflow. Coronary blood flow increased from 50±4 ml/min under control conditions to 170±5 ml/min during adenosine at rest ($P < 0.05$), and from 74±5 to 143±7 ml/min during exercise ($P < 0.05$) (Table VI). Adenosine tended to decrease the coronary pressure at zero flow during exercise, but this did not reach levels of statistical significance. In contrast to the high dose of pinacidil, adenosine increased coronary blood flow during exercise at coronary pressures as low as 30 mmHg, indicating that coronary vasodilator reserve was present even during myocardial hypoperfusion (Fig. 9).

Myocardial function. Adenosine had no effect on systolic wall thickening in either the LAD region or the control region at rest or during exercise. The downward limb of the coronary pressure–function tended to parallel the pressure–flow relation, but in the two animals this did not reach levels of statistical significance (not shown).

Discussion

The present study has yielded several new findings. (*a*) K^+_{ATP} channel blockade decreased coronary blood flow during exercise with normal and restricted arterial inflow. (*b*) Adenosine receptor blockade reduced coronary flow only at coronary pressures that were associated with severe myocardial hypoperfusion. (*c*) In contrast, in the presence of K^+_{ATP} channel blockade adenosine blockade aggravated coronary hypoperfusion, even at coronary pressures within the autoregulatory range. (*d*) K^+_{ATP} channel activation caused increments in coronary flow at normal but not at lower coronary pressures. (*e*) Adenosine produced an increase in flow during both normal and reduced coronary pressures. The implications of these findings will be discussed in detail.

Adenosine. Previous studies have demonstrated that endogenous adenosine is not obligatory for maintaining coronary blood flow during normal arterial inflow. Thus, studies in anesthetized open-chest dogs (15–19) and awake dogs (1, 20) failed to demonstrate an effect of intracoronary adenosine deaminase (15–17), or adenosine receptor blockade with intravenous aminophylline (18, 19) or 8-phenyltheophylline (1, 20), on

Table VI. Hemodynamic Data during Maximum Vasodilation with Adenosine

	Heart rate		Mean aortic pressure		LV systolic pressure		End-diastolic pressure		$LVdP/dt_{max}$		Coronary pressure		Coronary blood flow	
	Con	Ado	Con	Ado	Con	Ado	Con	Ado	Con	Ado	Con	Ado	Con	Ado
	beats/min		*mmHg*		*mmHg*		*mmHg*		*mmHg/s*		*mmHg*		*ml/min*	
Rest	120±8	125±8	86±5	85±5	109±5	111±4	7±1	7±1	2,330±130	2,690±120	86±5	62±2*	50±4	170±5*
Exercise	203±8‡	200±11‡	103±5‡	94±6	132±5‡	125±5‡	9±2	10±2‡	4,310±330‡	3,860±230‡	95±4	70±3*‡	74±5‡	143±7*‡
Exercise + occlusion	214±5‡	204±9‡	97±6‡	98±6‡	123±6‡§	127±5‡	23±1‡§	20±4‡§	4,120±420‡	3,830±220‡	26±3‡§	23±3‡§	0	0

Values are mean±SEM. $n = 7$; ‡ $P < 0.05$ vs rest, § $P < 0.05$ exercise vs exercise + occlusion, * $P < 0.05$ vs corresponding control measurements. *Con*, control; *Ado*, adenosine, 50 μg/kg per min i.c.

Figure 9. Coronary pressure–flow relation in dogs undergoing treadmill exercise. Shown are the relations during control (*open circles*) and adenosine, 50 μg/kg per min, intracoronary (*closed circles*). Data are mean±SEM, $n = 7$. *$P < 0.05$ versus corresponding control measurement.

basal coronary blood flow. McKenzie et al. (21) examined the effect of exercise on myocardial adenosine production. They found that treadmill exercise in dogs resulted in a fivefold increase in myocardial adenosine content, with a doubling of the coronary arterio-venous adenosine content difference. In dogs with chronically implanted pericardial catheters, graded exercise was associated with progressive increases of pericardial fluid adenosine concentrations (22–24), with a positive correlation between adenosine concentrations and coronary blood flow. However, Bache et al. (1) reported that adenosine receptor blockade with 8-phenyltheophylline or increased adenosine catabolism with adenosine deaminase did not alter exercise-induced increases in coronary blood flow. Taken together, these findings indicate that, although myocardial adenosine production increases during exercise, it is not obligatory for maintenance of coronary blood flow at rest or during exercise in the normal heart. This is in agreement with the present study, in which adenosine receptor blockade did not alter coronary blood flow at rest or during exercise with normal arterial inflow. In contrast, when coronary pressure was reduced, adenosine blockade significantly decreased blood flow during exercise. Similarly, adenosine receptor blockade has been previously reported to decrease coronary blood flow distal to a coronary artery stenosis (4, 5). These findings suggest that reductions of coronary artery pressure sufficient to cause myocardial hypoperfusion result in augmented adenosine production, which contributes to coronary vasodilation.

K^+_{ATP} channels. Several studies in anesthetized and awake dogs have investigated the role of K^+_{ATP} channels in maintaining coronary blood flow at rest and during exercise with normal arterial inflow. Using extracorporeally perfused canine hearts, Aversano et al. (8) found that intracoronary glibenclamide in doses of 0.8 and 3.7 μmol/min had no effect on basal coronary blood flow in open-chest dogs. 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 $\sim$ 14.4 and 66.5 μg/kg per min (mol $wt_{glibenclamide} = 494$), which are in the dose range used in the present study. In contrast, Imamura et al. (10) 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. We observed that glibenclamide in a dose of 50 μg/kg per min caused a 20–30% decrease in coronary blood flow in awake resting dogs (11, present study). In addition, coronary blood flow during exercise with normal arterial inflow was significantly decreased after K^+_{ATP} channel blockade. The reason for

the differences in the response of basal coronary flow to similar intracoronary doses of glibenclamide between our study and previous reports is not clear but might be related to the different experimental conditions, i.e., awake (11, present study) versus open-chest (10) versus open-chest extracorporeal perfusion (8).

Studies in anesthetized (10) and awake dogs (11) demonstrated that the decrease in coronary blood flow produced by K^+_{ATP} channel blockade with glibenclamide was associated with a decrease in regional systolic wall thickening. When coronary blood flow was restored to preglibenclamide levels with nitroprusside (which itself was devoid of any direct effect on systolic wall thickening), contractile performance recovered as well (10, 11), suggesting that glibenclamide caused a primary decrease in coronary flow with a secondary decrease in contractile function. Furthermore, the decrease in coronary blood flow caused by glibenclamide caused metabolic changes suggestive of ischemia, including a decrease in myocardial phosphorylation potential and creatine phosphate with an increase of inorganic phosphate in open-chest dogs (9). Thus, both mechanical and metabolic evidence support the concept that K^+_{ATP} channel blockade produced coronary vasoconstriction that resulted in ischemia-induced contractile dysfunction.

In the present study, K^+_{ATP} channel blockade decreased coronary blood flow both at coronary pressures in the autoregulatory range and at pressures that had resulted in hypoperfusion and loss of systolic wall thickening. These findings are in agreement with previous studies that indicate that K^+_{ATP} channels contribute not only to basal coronary tone but also to ischemic coronary vasodilation. Thus, studies in anesthetized (10) and awake dogs (11) have shown that K^+_{ATP} channel blockade not only decreases basal coronary blood flow but also blunts the reactive hyperemia in response to a brief coronary artery occlusion. Previous studies in open-chest dogs have suggested that progressively more K^+_{ATP} channels become activated as coronary pressure is decreased. Thus, using intravital microscopy and stroboscopic epiillumination, Komaru et al. (12) reported that superfusion of the subepicardial vasculature with glibenclamide (10^{-5} M) had no effect on the diameter of small arterioles (< 100 μm) at coronary artery pressures of 90–100 mmHg. In contrast, the arteriolar dilation that occurred in response to progressive coronary artery occlusion was abolished by glibenclamide. Similarly, in extracorporeally perfused dog hearts, intracoronary glibenclamide (10 μg/kg per min) had no effect on coronary blood flow at perfusion pressures equal to or greater than aortic pressure (> 100 mmHg) but significantly decreased flow at pressures < 80 mmHg (13). In contrast to these studies in open-chest anesthetized dogs, in the present study glibenclamide decreased coronary blood flow in intact exercising dogs both at coronary pressures below the autoregulatory range and at normal pressures, so that the absolute decrease in flow was similar at all coronary pressures. The data suggest that, although a significant number of K^+_{ATP} channels are open at coronary artery pressures in the autoregulatory range, progressively more channels are activated as pressure decreases. This hypothesis is supported by the finding that pinacidil increased coronary flow at normal pressures but not at pressures below the autoregulatory range, suggesting that, as perfusion pressure reaches the lower limit of autoregulation, most of the K^+_{ATP} channels have become activated. In contrast to the effects of pinacidil, adenosine increased flow at coronary pressures within but also below the autoregulatory range, suggesting that the lack of an increase in flow by pinacidil at very low perfusion pressures was not due to exhaustion of vasodilator reserve. Interestingly, Cobb and co-workers (25) reported that the addition of a dose of pinacidil that was without effect on basal coronary blood flow enhanced total reactive hyperemia flow after 30–60-s coronary artery occlusions, suggesting that brief coronary artery occlusions do not cause maximal recruitment of K^+_{ATP} channels.

Interaction between adenosine and K^+_{ATP} channels. In the present study, adenosine receptor blockade had no effect on coronary blood flow at rest or during exercise with normal arterial inflow. However, when the K^+_{ATP} channels were blocked with glibenclamide, adenosine blockade markedly aggravated myocardial hypoperfusion and further depressed myocardial systolic wall thickening. This suggests that endogenous adenosine released from the myocardium can oppose the coronary vasoconstrictor effect of glibenclamide. The finding that adenosine opposed the hypoperfusion produced by K^+_{ATP} channel blockade during exercise but not under resting conditions suggests that, after K^+_{ATP} channel blockade, exercise caused further 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 (26), who observed greater increases in interstitial adenosine concentrations in isovolumically beating hearts than in empty beating hearts when both underwent similar coronary blood flow reductions. Moreover, these authors observed excellent inverse correlations between the myocardial oxygen supply–demand balance and interstitial adenosine concentrations (26). Therefore, it is likely that, in the present study, impaired coronary vasodilation after K^+_{ATP} channel blockade caused further deterioration of the oxygen supply–demand balance during exercise. The consequent augmented release of adenosine into the myocardial interstitial space provided an alternate pathway for coronary vasodilation in response to exercise. This interpretation is supported by the report of Samaha et al. (9) that the addition of adenosine receptor blockade caused further deterioration of coronary blood flow and myocardial phosphorylation potential compared with K^+_{ATP} channel blockade alone in open-chest dogs.

Adenosine receptor blockade had no effect on coronary blood flow during exercise with normal arterial inflow, but decreased coronary flow at pressures below the autoregulatory plateau. In contrast, after glibenclamide, adenosine blockade further compromised myocardial perfusion during exercise at perfusion pressures both within and below the autoregulatory range. Thus, while in the presence of unimpaired K^+_{ATP} channel activity, adenosine plays a role in the regulation of coronary vasomotor tone only at lower coronary pressures, adenosine also becomes important for the control of flow at coronary pressures in the autoregulatory range when K^+_{ATP} channels are blocked.

Mechanism of interaction between adenosine and K^+_{ATP} channels. In the present study, the increases in coronary blood flow 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 coronary blood flow produced by nitroprusside was not altered by either glibenclamide or 8-phenyltheophylline, indicating that neither agent caused nonspecific blunting of vasodilator responsiveness (27). The coronary va-

sodilation produced by the K^+_{ATP} channel opener pinacidil was selectively inhibited by glibenclamide but was not affected by 8-phenyltheophylline. In contrast, the vasodilation produced by adenosine was attenuated by both 8-phenyltheophylline and by glibenclamide. This observation is in agreement with previous reports that adenosine produces coronary vasodilation via activation of K^+_{ATP} channels (8, 28, 29). Although glibenclamide inhibited the dilation caused by intracoronary adenosine, endogenous adenosine continued to exert a vasodilator effect during exercise even in the presence of glibenclamide. 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. It has been proposed that, under conditions of normal coronary arterial inflow, an increase in metabolic demands causes opening of vascular smooth muscle K^+_{ATP} channels (7). Although regulation of these channels in vascular smooth muscle is incompletely understood, a decrease in ATP or an increase in ADP levels near the sarcolemma of vascular smooth muscle cells (perhaps in response to decreased periarteriolar oxygen tension) is believed to activate these channels (7). Adenosine receptors, which can activate K^+_{ATP} channels via a G-protein (7), 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 coronary blood flow (1). However, when vascular smooth muscle 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. The present findings suggest that, when 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. It is possible that, when exercise in the presence of glibenclamide causes further deterioration of the myocardial oxygen supply–demand balance, adenosine concentrations increase to levels that can effectively compete with glibenclamide, resulting in activation of the channels and coronary vasodilation (26, 30).

It is also 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 via stimulation of vascular smooth muscle adenosine A_2 receptors, which activate adenylyl cyclase (31–33). In support of this hypothesis, in the present study, glibenclamide was slightly more effective at blocking the vasodilation produced by pinacidil than that caused by adenosine, suggesting that adenosine could have mediated part of its effect through another vasodilator pathway, likely adenylyl cyclase activation. Furthermore, pinacidil failed to produce an increase in coronary flow at very low perfusion pressures, whereas adenosine increased flow at coronary pressures both within and below the autoregulatory range. These findings indicate that adenosine produced coronary vasodilation in part via K^+_{ATP} channel activation but also via other mechanisms.

Methodological considerations. The coronary pressure–flow relation describes the blood flow response over a range of perfusion pressures within and below the autoregulatory range. The behavior of coronary blood flow over a range of perfusion pressures is relevant to the clinical situation in which an arterial stenosis can result in decreased perfusion pressure. Determination of the entire pressure–flow relation allows examination of blood flow responses over a wide range of poststenotic pressures produced by varying degrees of coronary artery stenosis, allowing a comprehensive assessment of regulation of coronary vasomotor tone. In addition to coronary vasomotor tone, other factors can influence coronary blood flow at a given arterial pressure. Extravascular compressive forces that act on the coronary vasculature can impede coronary blood flow; these forces are highest during systole, when the contracting myocardium compresses the intramural vasculature (34). Consequently, an increase in heart rate, which results in an increase in the relative time spent in systole each cardiac cycle, will result in an increase in averaged extravascular compressive forces (34, 35). In addition, left ventricular intracavitary pressure during diastole can impede coronary blood flow. Therefore, to be able to interpret the changes in coronary artery blood flow as reflections of changes in coronary vasomotor tone, it is mandatory that systemic hemodynamic variables are similar between sequential exercise trials. In the present study, hemodynamic variables (in particular left ventricular end-diastolic pressure and heart rate) were very similar between exercise trials, indicating that differences in systemic hemodynamic profile cannot account for the observed differences in coronary blood flow.

Another factor that can influence the coronary artery pressure–flow relation is collateral blood flow. As coronary artery pressure distal to the stenosis drops, collateral blood flow can contribute to total myocardial tissue flow, resulting in underestimation of myocardial tissue flow when measuring blood flow on a proximal coronary artery. In the present study, we did not measure myocardial tissue blood flow. However, Messina et al. (36) reported that collateral blood flow begins to contribute significantly to myocardial tissue blood flow when intercoronary pressure gradients exceed 70 mmHg. In the present study, during exercise mean aortic pressure was in the range of 100–110 mmHg while the pressure at zero flow was in the range of 30–40 mmHg, which would suggest that the collateral driving pressure was probably not sufficient to result in a considerable contribution of collateral blood flow to total myocardial tissue flow. Furthermore, the observation that changes in coronary pressure–flow relation produced by K^+_{ATP} channel (ant)agonists and adenosine receptor (ant)agonists were accompanied by changes in the coronary–function relation (which is determined by myocardial tissue perfusion) indicates that the changes in the coronary pressure–flow relation measured on a proximal coronary artery did reflect changes in myocardial tissue blood flow, and were not compensated for by an increase in collateral blood flow. Finally, the finding that the coronary pressure–flow and pressure–function relations were nearly identical during three consecutive control runs without a rightward shift of the relations also indicates that there was no significant collateral blood flow recruitment after the first exercise trial.

In the present study, we did not measure the transmural distribution of myocardial blood flow. Consequently, we cannot determine whether the changes in coronary blood flow produced by the various agonists and antagonists occurred in the inner or outer layers of the left ventricular wall. Gallagher et al. (37) demonstrated that full wall thickening depends on

subendocardial perfusion and correlates poorly with subepicardial perfusion, suggesting that the decreases in blood flow produced by glibenclamide and 8-phenyltheophylline in the present study, which were paralleled by decreases in systolic wall thickening, were also present in the innermost layers. This is also supported by our previous studies that demonstrated that adenosine receptor blockade aggravates hypoperfusion in all myocardial layers distal to a coronary artery stenosis during exercise (4, 5). The observation in the present study that, after K^+_{ATP} channel blockade with glibenclamide, systolic wall thickening was depressed proportionally more than coronary artery blood flow could be interpreted to suggest that a nonuniform blood flow distribution could have occurred with a more prominent decrease in subendocardial than in subepicardial blood flow. We cannot determine from the present study whether the increase in coronary blood flow produced by adenosine at low perfusion pressures was functionally significant, as systolic wall thickening could be measured in only two of the six animals. However, we previously observed that, in the presence of a coronary artery stenosis which resulted in a coronary artery pressure of 43±2 mmHg and caused hypoperfusion in all myocardial layers during exercise, an intracoronary infusion of adenosine increased flow to all myocardial layers and resulted in an increase in systolic segment shortening (38). These findings suggest that the changes in flow produced by glibenclamide, 8-phenyltheophylline, and adenosine are functionally significant and thus reflect, at least in part, changes in subendocardial blood flow.

In the present study, we demonstrated that glibenclamide produced a high degree of K^+_{ATP} channel blockade without causing nonspecific blunting of vasodilator responsiveness as indicated by a preserved nitroprusside vasodilator response. However, glibenclamide can also block other K^+ channels in some tissues. Thus, in rat portal vein cell preparation, glibenclamide inhibited K^+_{ATP} channels but also K^+_{Ca2+} channels (39). However, in porcine coronary vascular smooth cells (40), the intact arterial bed of the canine diaphragm (41), or the isolated rat heart (42), there was no overlap in the pharmacological actions of glibenclamide and known blockers of K^+_{Ca2+} channels, including large and small conductance K^+_{Ca2+} channels (charybdotoxin, iberiotoxin, apamin), blockers of $K^+_{voltage}$ channels (charybdotoxin), or blockers of delayed rectifier K^+ currents (E-1403). These data suggest that in most tissues, including the coronary vascular bed, glibenclamide is a selective K^+_{ATP} channel blocker.

Conclusions. K^+_{ATP} channel blockade decreased coronary blood flow during exercise at coronary pressures within and below the autoregulatory range, indicating coronary K^+_{ATP} channel activity during exercise with both normal arterial inflow and restricted inflow distal to a coronary artery stenosis. Adenosine receptor blockade had no effect on coronary flow at pressures within the autoregulatory range but decreased flow at low perfusion pressures that were associated with severe myocardial hypoperfusion. In contrast, in the presence of K^+_{ATP} channel blockade, the addition of adenosine receptor blockade further decreased coronary flow even at coronary pressures within the autoregulatory range, indicating increased importance of the vasodilator influence of endogenous adenosine during exercise when K^+_{ATP} channels are blocked. Intracoronary adenosine increased coronary flow at coronary pressures both within and below the autoregulatory range. In contrast, selective K^+_{ATP} channel activation with pinacidil increased flow only at normal coronary pressures, suggesting that most K^+_{ATP} channels are recruited as perfusion pressures reach the lower limit of autoregulation. These findings indicate that K^+_{ATP} channels and endogenous adenosine play a synergistic role in maintaining coronary blood flow during exercise in normal hearts and at the low perfusion pressures that can exist distal to a coronary artery stenosis.

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