Role of K⁺ATP Channels in Coronary Vasodilation During Exercise

Dirk J. Duncker, MD, PhD; Noëmi S. Van Zon, MS; John D. Altman, MD; Todd J. Pavek, BA; Robert J. Bache, MD

Background. The mechanism of metabolic regulation of coronary vascular tone is still unclear. Therefore, we examined the role of vascular smooth muscle K⁺ATP channels in regulating coronary blood flow under resting conditions, during increments in myocardial metabolic demand produced by treadmill exercise, and in response to a brief ischemic stimulus.

Methods and Results. Ten chronically instrumented dogs were studied at rest and during a four-stage exercise protocol under control conditions and during intracoronary infusion of the K⁺ATP channel blocker glybenclamide at rates of 10 and 50 μg·kg⁻¹·min⁻¹. Glybenclamide (50 μg·kg⁻¹·min⁻¹) decreased coronary blood flow at rest from 51±4 to 42±6 mL/ min (P<.05), decreased myocardial oxygen consumption from 5.70±0.31 to 4.11±0.56 mL O₂/min (P<.05), and decreased systolic wall thickening from 21±3% to 12±3% (P<.05). The depression of systolic wall thickening produced by glybenclamide was reversed when coronary blood flow was restored to the control level with intracoronary nitroprusside, indicating a primary effect of glybenclamide on coronary flow during resting conditions. However, glybenclamide did not impair the increases of coronary blood flow, myocardial oxygen consumption, and systolic wall thickening that occurred during exercise. In eight resting awake dogs, 50 μg·kg⁻¹·min⁻¹ glybenclamide decreased the peak reactive hyperemia blood flow rate following a 20-second coronary occlusion from 149±14 mL/min during control conditions to 111±15 mL/min (P<.05), decreased the duration of reactive hyperemia from 49±6 to 33±3 seconds (P<.05), and decreased reactive hyperemia excess flow from 33±5 to 20±4 mL (P<.05).

Conclusions. These data demonstrate that K⁺ATP channels modulate coronary vasomotor tone under resting conditions and contribute to coronary vasodilatation during ischemia. However, the coronary vasculature retains the capacity to dilate in response to increases in oxygen demand produced by exercise when K⁺ATP channels are blocked. (Circulation. 1993;88:1245-1253.)

KEY WORDS • blood flow • vasoconstriction • ischemia • exercise • potassium

In the normal heart, coronary blood flow is precisely regulated in response to changing myocardial needs to maintain a consistently high level of oxygen extraction by the myocardium.¹ This close coupling between myocardial demands and coronary blood flow is especially apparent during the coronary vasodilation that occurs with exercise. The linkage of coronary vasomotor tone to myocardial metabolic demands has been suggested to depend on messengers released from the myocardium or vascular endothelium.² ³ However, specific blockers of known endogenous vasodilators such as adenosine, prostacyclin, or nitric oxide have not been found to impair the normal increases in coronary blood flow that occur during exercise.⁴ ⁶

Recent evidence indicates that hyperpolarization of coronary vascular smooth muscle caused by opening of K⁺ATP channels contributes to regulation of coronary vasomotor tone.⁷ ⁹ Thus, agents that inhibit K⁺ATP channel opening have been reported to decrease basal levels of coronary blood flow and impair coronary vasodilation in response to hypoxia and during normal autoregulation.¹⁰ ¹² These findings suggest that opening of K⁺ATP channels might also contribute to coronary vasodilatation during exercise. Consequently, this study was carried out to determine whether K⁺ATP channel blockade with glybenclamide would impair exercise-induced coronary vasodilatation in the chronically instrumented dog.

Methods

Studies were performed in 15 adult mongrel dogs weighing 23 to 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 under the supervision of the Animal Care Committee of the University of Minnesota.

Surgical Preparation

Following sedation with fentanyl (0.4 mg IM) and droperidol (20 mg IM), dogs were anesthetized with sodium pentobarbital (30 to 35 mg/kg IV), intubated, and ventilated with a mixture of oxygen (30%) and room air (70%). Respiratory rate and tidal volume were set to keep arterial blood gases within physiological limits. A left thoracotomy was performed through the fifth inter-
costal space, and the heart was suspended in a pericardial cradle. A polyvinyl chloride catheter (3.0 mm outer diameter) 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 Instrument Co, Pasadena, Calif) 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 was dissected free, and a Doppler flow probe (Craig Hartley, Houston, Tex) was positioned around the artery. Immediately distal to the flow probe, a hydraulic occluder (3.0 mm outer diameter) 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 left anterior descending coronary artery 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 left anterior descending coronary artery and the left circumflex coronary artery (control area), respectively. In five 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 left anterior descending coronary artery. 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 was evacuated. Catheters were flushed daily with heparinized saline.

**Hemodynamic Measurements**

Studies were performed 2 to 3 weeks after surgery with animals exercising on a motor-driven treadmill or standing in a sling. Recordings of phasic and mean aortic pressures were measured with Gould P23XL pressure transducers positioned at midchest 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 blood velocity was measured with a Doppler flowmeter system (Craig Hartley, Houston, Tex). Data were recorded on an eight-channel direct-writing oscillograph (Coulbourn Instruments, Lehigh Valley, Pa).

**Regional Myocardial Function Measurements**

Regional systolic wall thickening was measured by sonomicrometry (model 120, Triton Technology, San Diego, Calif) 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 two animals and in the left anterior coronary artery segment in one animal. End-diastolic wall thickness (EDT) was measured at the onset of positive LVdP/dt and end-systolic wall thickness was measured 20 milliseconds before peak negative LVdP/dt. Percent myocardial systolic wall thickening (SWT) was calculated as:

\[
\text{SWT} \% = \frac{(\text{EST} - \text{EDT}) \times \text{EDT}}{100}
\]

**Experimental Protocols**

Magnitude and selectivity of \(K_{ \text{ATP}}\) channel blockade with glybenclamide. The effects of glybenclamide on the increases in coronary blood flow caused by the \(K_{ \text{ATP}}\) channel opener pinacidil and the \(K_{ \text{ATP}}\) channel–independent coronary vasodilator nitroprusside were studied in four dogs. Initial baseline measurements of systemic hemodynamics and coronary blood flow were made with the dogs resting quietly in a sling. Then, the coronary blood flow responses to intracoronary infusions of pinacidil in doses of 0.25, 0.5, 1.0, and 2.5 \(\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) (infusion rate, 0.15 to 1.5 mL/min) were assessed. After washout of pinacidil, an intracoronary infusion of glybenclamide was started in a dose of 10 \(\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) (infusion rate, 0.3 mL/min). Glybenclamide was dissolved in dimethyl sulfoxide and saline to which sodium bicarbonate was added to bring the pH to 8.5. Five minutes after beginning the infusion of glybenclamide, coronary blood flow responses to intracoronary pinacidil (0.5, 1.0, and 2.5 \(\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) ) were again recorded. The infusion rate of glybenclamide was then increased to 50 \(\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\), and the responses to pinacidil in doses of 0.5, 1.0, 2.5, and 5 \(\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) were observed. To determine whether glybenclamide caused a nonspecific alteration in vascular responsiveness, we examined the response of coronary blood flow to intracoronary infusions of nitroprusside on a separate day. Sodium nitroprusside dihydrate dissolved in sterile water and pro-

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**TABLE 1. Hemodynamic Data From 10 Dogs at Rest and During Graded Treadmill Exercise**

<table>
<thead>
<tr>
<th>Exercise (speed/grade)</th>
<th>Heart rate (bpm)</th>
<th>Mean aortic pressure (mm Hg)</th>
<th>Left ventricular systolic pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>G10</td>
<td>G50</td>
</tr>
<tr>
<td>Rest</td>
<td>129±12</td>
<td>134±11</td>
<td>149±10†</td>
</tr>
<tr>
<td>Exercise (km/h, %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.8 km/h, 0%</td>
<td>181±9*</td>
<td>181±8</td>
<td>190±7</td>
</tr>
<tr>
<td>6.4 km/h, 5%</td>
<td>201±7*</td>
<td>208±6</td>
<td>210±5</td>
</tr>
<tr>
<td>6.4 km/h, 10%</td>
<td>214±7*</td>
<td>223±7</td>
<td>224±7</td>
</tr>
<tr>
<td>6.4 km/h, 20%</td>
<td>244±7*</td>
<td>247±7</td>
<td>241±7</td>
</tr>
</tbody>
</table>

CON indicates control; G10, glybenclamide 10 \(\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) IC; and G50, glybenclamide 50 \(\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) IC. Values are mean±SEM.

*P<.05 vs rest; †P<.05 vs corresponding control measurement.
tected from light was infused into the coronary artery catheter in doses of 0.6, 1.5, and 3.0 μg·kg⁻¹·min⁻¹ (infusion rate, 0.06 to 0.3 mL/min). Responses to nitroprusside were studied during control conditions with and with glybenclamide infusions of 10 and 50 μg·kg⁻¹·min⁻¹.

**Reactive hyperemia protocol.** The effects of glybenclamide on the reactive hyperemic responses to coronary artery occlusions were studied in eight dogs. With dogs resting quietly in a sling, baseline measurements were made of systemic hemodynamics and coronary blood flow. Then reactive hyperemic responses to coronary occlusions 5, 10, and 20 seconds in duration were recorded in duplicate. A 3-minute interval was allowed between occlusions. Subsequently, glybenclamide in a dose of 10 μg·kg⁻¹·min⁻¹ was infused into the coronary artery catheter (infusion rate, 0.3 mL/min). Five minutes after beginning the glybenclamide infusion, hemodynamic measurements were obtained, and the reactive hyperemic responses to coronary artery occlusions of 5, 10, and 20 seconds' duration were again observed. This sequence was repeated in the presence of glybenclamide infused into the coronary catheter in a dose of 50 μg·kg⁻¹·min⁻¹ (infusion rate, 1.5 mL/min).

**Exercise protocol.** After all recording instruments were connected, animals were allowed to rest on the treadmill for 30 minutes. After steady-state conditions were achieved, hemodynamic and functional measurements as well as arterial and coronary venous blood specimens were obtained under resting conditions. A 3-minute period of warm-up exercise was then begun at a treadmill speed of 3.2 km/h at 0% grade. Fifteen minutes later, a four-stage treadmill exercise protocol as shown in Table 1 was begun. Each exercise stage was 2 to 3 minutes in duration; left ventricular and aortic blood pressure, coronary blood flow, and myocardial function were measured, and blood specimens were obtained during the last 30 seconds of each exercise stage, when hemodynamics had reached a steady state.

After a 90-minute rest period, an infusion of glybenclamide (10 μg·kg⁻¹·min⁻¹) was begun into the coronary artery catheter delivered at a rate of 0.3 mL/min. Five minutes after beginning the infusion, resting measurements were obtained, and the four-stage exercise protocol was repeated. The infusion was discontinued, and the animals allowed to rest for 90 minutes. Then, the intracoronary infusion of glybenclamide was restarted at a rate of 1.5 mL/min, equivalent to 50 μg·kg⁻¹·min⁻¹, and the exercise protocol was repeated.

Blood specimens were maintained in iced syringes until the conclusion of each exercise protocol. Measurements of PO₂, PCO₂, and pH then were immediately

**TABLE 1.** Continued

<table>
<thead>
<tr>
<th>End-diastolic pressure (mm Hg)</th>
<th>Left ventricular dP/dt max (mm Hg/s)</th>
<th>Coronary blood flow (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CON</strong></td>
<td><strong>G10</strong></td>
<td><strong>G50</strong></td>
</tr>
<tr>
<td>5.5±0.5</td>
<td>7.6±1.1</td>
<td>9.6±0.8†</td>
</tr>
<tr>
<td>8.7±0.9*</td>
<td>8.2±1.1</td>
<td>13.7±1.5†</td>
</tr>
<tr>
<td>10.2±1.4*</td>
<td>10.8±1.8</td>
<td>16.7±2.1†</td>
</tr>
<tr>
<td>10.2±0.9*</td>
<td>11.6±1.6</td>
<td>16.4±2.1†</td>
</tr>
<tr>
<td>11.5±1.0*</td>
<td>14.6±2.4</td>
<td>18.8±2.3†</td>
</tr>
</tbody>
</table>

**FIG 1.** Plots of effects of glybenclamide on the increases in coronary blood flow (CBF) caused by pinacidil (left) and sodium nitroprusside (right) in four dogs. Shown are the blood flow responses under control conditions (●), glybenclamide 10 μg·kg⁻¹·min⁻¹ (▲), and glybenclamide 50 μg·kg⁻¹·min⁻¹ (●).
Doppler flowmetry was performed using the method described by Ishida and colleagues. Total blood flow during reactive hyperemia was determined by electrical integration of the Doppler shift tracing. Reactive hyperemia data were analyzed as: reactive hyperemia excess flow equals total flow during reactive hyperemia (mL) minus control flow rate (mL/s) multiplied by duration of reactive hyperemia (s). In the exercise protocol, coronary blood flow was analyzed as a function of heart rate and as a function of a calculated index of time-averaged systolic wall stress. The latter was calculated as the product of heart rate (HR) and left ventricular systolic blood pressure (LVSP) divided by the average systolic wall thickness. The former 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 ANOVA for repeated measures. When a significant effect was observed, individual comparisons were made using the Wilcoxon signed-rank test, with a modified Bonferroni correction for multiple comparisons. The effect of glybenclamide on the relationship between two variables was analyzed by multivariate regression analysis. Statistical significance was accepted at P<.05 (two-tailed). All data are presented as mean±SEM.

Results

Magnitude and Selectivity of \( K_{ATP}^+ \) Channel Blockade by Glybenclamide

The effect of glybenclamide on the increases in coronary blood flow caused by pinacidil and nitroprusside are shown in Fig 1. Glybenclamide caused dose-dependent inhibition of the coronary vasodilation in response to pinacidil (up to 90% inhibition with high-dose glybenclamide) but had no effect on the response to nitroprusside. These findings indicate that glybenclamide produced a high degree of \( K_{ATP}^+ \) inhibition.

![Graph] (Fig 2. An example of a typical reactive hyperemia in response to a 10-second coronary artery occlusion before and during intracoronary (ic) infusion of glybenclamide. CBF indicates coronary blood flow.)

| Table 2. Reactive Hyperemia After Occlusions of Left Anterior Descending Coronary Artery of 5-, 10-, and 20-Second Duration in Eight Dogs |
|-----------------|-----------------|-----------------|
|                 | 5-Second        | 10-Second       | 20-Second       |
|                 | CON  | G10  | G50  | CON  | G10  | G50  | CON  | G10  | G50  |
| CBF basal (mL/min) | 37±5 | 34±5 | 30±5* | 37±5 | 32±5 | 30±5* | 37±6 | 34±5 | 28±5* |
| CBF peak (mL/min)  | 117±13 | 106±13 | 78±11* | 142±15 | 124±15* | 104±15* | 149±14 | 137±16* | 111±15* |
| Duration (seconds) | 16.7±1.3 | 13.7±2.6 | 12.0±2.0* | 36±4 | 23±2* | 23±3* | 49±6 | 36±4* | 33±3* |
| Excess flow (mL)   | 8.9±1.9 | 6.3±1.2* | 4.1±0.7* | 17.8±2.8 | 13.0±2.3* | 11.7±2.7* | 33.2±5.1 | 24.9±3.9* | 19.7±4.0* |

CBF indicates coronary blood flow; CON, control; G10, glybenclamide 10 \( \mu g \cdot kg^{-1} \cdot min^{-1} \) IC; and G50, glybenclamide 50 \( \mu g \cdot kg^{-1} \cdot min^{-1} \) IC. Values are mean±SEM.

*P<.05 compared with corresponding control measurement.
channel blockade without impairing other vasodilator mechanisms.

Reactive Hyperemia

During quiet resting conditions, heart rate and mean aortic blood pressure were 105±9 beats per minute and 92±2 mm Hg, respectively. These measurements were not significantly altered by intracoronary infusions of glybenclamide in a dose of either 10 μg·kg⁻¹·min⁻¹ (106±8 beats per minute and 90±3 mm Hg) or 50 μg·kg⁻¹·min⁻¹ (106±8 beats per minute and 97±3 mm Hg). An example of a reactive hyperemic response is shown in Fig 2. Glybenclamide decreased coronary blood flow under resting conditions, and this reached statistical significance at the higher dose (Table 2). Peak blood flow rates during reactive hyperemia following 5-, 10-, and 20-second occlusions were attenuated by glybenclamide in a dose-dependent manner. The absolute reduction in peak reactive hyperemia blood flow produced by glybenclamide was independent of the duration of the coronary artery occlusion (Table 2, Fig 3). The duration of reactive hyperemia was markedly shortened by glybenclamide. Interestingly, the effects of the two doses of glybenclamide were significantly different with respect to the peak flow (P<.05 for all occlusion durations) but were similar with respect to reactive hyperemia duration (Table 2). Reactive hyperemia excess flow was decreased by glybenclamide in both a dose-dependent and an occlusion duration–dependent manner (Table 2, Fig 3).

Exercise

The hemodynamic responses to increasing levels of exercise are shown in Table 1. Heart rate increased from 129±12 at rest to 244±7 beats per minute at peak exercise (P<.05), mean arterial pressure from 94±4 to 116±6 mm Hg (P<.05), left ventricular systolic pressure from 116±4 to 151±4 mm Hg (P<.05), left ventricular end-diastolic pressure from 5.5±0.5 to 11.5±1.0 mm Hg (P<.05), and left ventricular dp/dt max from 2770±130 to 6470±590 mm Hg (P<.05) (Table 1). Coronary blood flow increased from 51±4 mL/min at rest to 99±7 mL/min at the highest level of exercise (P<.05). Glybenclamide at 10 μg·kg⁻¹·min⁻¹ had no significant effect on any of the systemic hemodynamic variables. However, coronary blood flow was significantly lower at rest and during each level of exercise. Glybenclamide at 50 μg·kg⁻¹·min⁻¹ further decreased coronary blood flow; this was associated with significant elevations of left ventricular end-diastolic pressure at rest and at each level of exercise and a significant reduction of left ventricular dp/dt max at the highest level of exercise.

The reductions in coronary blood flow were not due to glybenclamide-induced decreases in the indices of myocardial oxygen demand. This is illustrated by Fig 4, in which coronary blood flow has been plotted against heart rate and an index of systolic wall stress. For a given level of heart rate or systolic wall stress index, coronary blood flow was reduced by glybenclamide in a dose-dependent manner. Both doses of glybenclamide resulted in parallel rightward shifts of the relationships between coronary blood flow and both heart rate and the index of systolic wall stress, with no significant change in the slope of these relationships. When coronary blood flow was expressed as a percent of the resting level, glybenclamide did not significantly impair the relative exercise-induced increases in blood flow.

In five animals, blood samples were obtained from the aorta and the great cardiac vein for determination of myocardial oxygen consumption. In these five animals, during control conditions coronary blood flow increased from 48±4 at rest to 101±9 mL/min at the highest level
FIG 6. Plot of relationship between coronary blood flow (CBF) and the oxygen content difference between arterial and coronary venous blood (A-V O₂ diff) in five dogs at rest and during incremental levels of treadmill exercise. Shown are the relationships under control conditions ( ), glybenclamide 10 μg·kg⁻¹·min⁻¹ ( ), and glybenclamide 50 μg·kg⁻¹·min⁻¹ ( ). 

For the sake of clarity, the standard error bars have been omitted from the data points obtained during glybenclamide 10 μg·kg⁻¹·min⁻¹. *P<.05 vs control.

of exercise (P<.05), whereas myocardial oxygen consumption increased from 5.7±0.3 to 13.4±0.6 mL O₂/min (P<.05). Glybenclamide caused a slightly greater decrease in coronary blood flow than in myocardial oxygen consumption (Fig 5), as the glybenclamide-induced flow reductions were accompanied by an increase in oxygen extraction (Fig 6).

Exercise had no effect on end-diastolic left ventricular wall thickness but increased end-systolic wall thickness in both the left anterior descending coronary artery perfused area (Table 3) and the control area (not shown). Consequently, percent systolic wall thickening increased from 21±3% at rest to 31±3% (P<.05) in the area perfused by the left anterior descending coronary artery and from 21±3% to 29±4% (P<.05) in the control area. Glybenclamide had no effect on the end-diastolic wall thickness but decreased end-systolic thickness at rest and during each level of exercise in a dose-dependent manner (Table 3). Percent systolic wall thickening in the area perfused by the left anterior descending coronary artery was significantly reduced by glybenclamide. The magnitude of the decrease in systolic wall thickening produced by glybenclamide was independent of the exercise level (Table 3, Fig 7). Glybenclamide had no effect on the response of systolic wall thickening to exercise in the control region perfused by the left circumflex coronary artery (Fig 7).

FIG 7. Plots of relationship between heart rate (HR) and percent systolic wall thickening (SWT) of the areas perfused by the left anterior descending coronary artery (LAD) (n=9) (left) and the control area (n=8) (right) in dogs at rest and during incremental levels of treadmill exercise. Shown are the relationships under control conditions ( ), glybenclamide 10 μg·kg⁻¹·min⁻¹ ( ), and glybenclamide 50 μg·kg⁻¹·min⁻¹ ( ). Intracoronary infusions of glybenclamide caused a dose-dependent downward shift of the relationship in the LAD area, whereas in the control area perfused by the left circumflex coronary artery (LCX), the relationship was unaltered. *P<.05 vs control.

Effect of Vehicle

The effects of the glybenclamide vehicle on the exercise-induced changes in coronary blood flow and systolic wall thickening were studied in five dogs. As shown in Fig 8, the vehicle had no effect on either blood flow or systolic wall thickening in the area perfused by the left anterior descending coronary artery.

Mechanism of Depressed Systolic Function

To determine whether the decrease in systolic wall thickening after glybenclamide resulted from impaired myocardial perfusion, the effect of returning coronary blood flow to the preglybenclamide level was examined during resting condition in three dogs. As shown in Table 4, during control conditions intracoronary infusion of nitroprusside (10 μg·kg⁻¹·min⁻¹) increased coronary blood flow without an effect on systolic wall thickening. Glybenclamide (10 and 50 μg·kg⁻¹·min⁻¹ intracoronary) decreased both coronary blood flow and systolic wall thickening. While the infusion of glybenclamide continued, the addition of nitroprusside restored regional function as it increased blood flow. This supports the hypothesis that the glybenclamide-associated decrease of regional contractile function occurred secondary to the reduction in coronary blood flow.

<p>| TABLE 3. Contractile Function of the Myocardium Perfused by the Left Anterior Descending Coronary Artery Obtained From Nine Dogs at Rest and During Graded Treadmill Exercise |
|---------------------------------|--------|--------|--------|--------|--------|--------|
| End-diastolic thickness (mm)    | End-systolic thickness (mm) | Systolic wall thickening (%) |</p>
<table>
<thead>
<tr>
<th>CON</th>
<th>G10</th>
<th>G50</th>
<th>CON</th>
<th>G10</th>
<th>G50</th>
<th>CON</th>
<th>G10</th>
<th>G50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>7.2±0.8</td>
<td>7.1±0.8</td>
<td>6.9±0.8</td>
<td>8.6±0.8</td>
<td>8.3±0.9†</td>
<td>7.7±0.7†</td>
<td>21±3</td>
<td>17±4</td>
</tr>
<tr>
<td>Exercise (speed/grade)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.8 km/h, 0%</td>
<td>7.2±0.8</td>
<td>7.1±0.8</td>
<td>7.0±0.8</td>
<td>8.8±0.9*</td>
<td>8.4±0.9†</td>
<td>7.8±0.8†</td>
<td>23±3*</td>
<td>19±3†</td>
</tr>
<tr>
<td>6.4 km/h, 5%</td>
<td>7.1±0.8</td>
<td>7.1±0.8</td>
<td>6.9±0.8</td>
<td>8.8±0.9*</td>
<td>8.5±0.9†</td>
<td>7.9±0.8†</td>
<td>25±3*</td>
<td>20±3†</td>
</tr>
<tr>
<td>6.4 km/h, 10%</td>
<td>7.1±0.8</td>
<td>7.1±0.8</td>
<td>6.9±0.8</td>
<td>8.9±0.9†</td>
<td>8.6±0.9†</td>
<td>7.9±0.8†</td>
<td>27±3*</td>
<td>22±3†</td>
</tr>
<tr>
<td>6.4 km/h, 20%</td>
<td>7.1±0.8</td>
<td>7.1±0.8</td>
<td>6.9±0.8</td>
<td>9.2±0.9*</td>
<td>8.9±0.9†</td>
<td>8.1±0.8†</td>
<td>31±3*</td>
<td>27±3†</td>
</tr>
</tbody>
</table>

CON indicates control; G10, glybenclamide 10 μg·kg⁻¹·min⁻¹ IC; and G50, glybenclamide 50 μg·kg⁻¹·min⁻¹ IC. Values are mean±SEM.

*P<.05 vs rest; †P<.05 vs corresponding control measurement.
canine hearts. In a preliminary report, Imamura and colleagues\textsuperscript{16} found that infusion of glybenclamide in a dose of 50 \(\mu g \cdot kg^{-1} \cdot min^{-1}\) caused a 40\% decrease in basal coronary blood flow and decreased reactive hyperemia excess flow by as much as 80\% in intact dogs. In the present study, the same dose of glybenclamide caused a 20\% decrease in basal coronary blood flow and decreased the reactive hyperemia flow by approximately 50\%. These studies demonstrate that \(K_{\text{ATP}}\) channels contribute importantly to postischemic reactive hyperemic responses in both perfused and intact hearts.

Samaha and colleagues\textsuperscript{11} used intracoronary infusions of glybenclamide in open-chest dogs to achieve blood concentrations of 20 to 80 \(\mu M\). These doses of glybenclamide correspond to infusion rates of 16 to 64 \(\mu g \cdot kg^{-1} \cdot min^{-1}\), which are in the range used in the present study. Glybenclamide (80 \(\mu M\)) decreased coronary blood flow to 50\% of baseline, but myocardial oxygen consumption was not decreased because oxygen extraction nearly doubled. Such large increases in myocardial oxygen extraction can occur in open-chest preparations where general anesthesia and acute surgical trauma have decreased basal coronary tone but are not possible under physiological conditions in the awake animal where oxygen extraction commonly exceeds 80\% during resting conditions (81\%\pm 3\%) during resting conditions in the present study). Although the decrease in coronary blood flow produced by glybenclamide in the study of Samaha and colleagues\textsuperscript{11} failed to cause myocardial oxygen consumption to decrease, the investigators observed conversion from lactate consumption to production, suggesting that oxygen availability to the myocardium was impaired.

In the present study, glybenclamide decreased coronary blood flow at rest. Although myocardial oxygen extraction increased, the high basal level of oxygen extraction prevented full compensation for the decrease in coronary blood flow, resulting in a significant decrease of oxygen delivery. The decreased oxygen availability was associated with contractile dysfunction. In three conscious dogs, we observed that glybenclamide-induced myocardial hypofunction was reversed when coronary blood flow rates were restored to preglybenclamide levels with sodium nitroprusside, confirming earlier observations in anesthetized dogs.\textsuperscript{19} These findings, together with the observation that potassium channel activators can exert negative inotropic effects,\textsuperscript{5,20} strongly suggest that the glybenclamide-in-

### Table 4. Effect of Increasing Coronary Blood Flow by Intra-arterial Infusion of Nitroprusside on Regional Systolic Wall Thickening in Three Dogs During Control Conditions and During Intracoronary Infusion of Glybenclamide

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>NP</th>
<th>G10</th>
<th>G10+NP</th>
<th>G50</th>
<th>G50+NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>93\pm 5</td>
<td>94\pm 14</td>
<td>79\pm 5</td>
<td>99\pm 14</td>
<td>90\pm 5</td>
<td>101\pm 11</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>105\pm 6</td>
<td>104\pm 7</td>
<td>104\pm 9</td>
<td>100\pm 13</td>
<td>104\pm 9</td>
<td>101\pm 7</td>
</tr>
<tr>
<td>Coronary blood flow (mL/min)</td>
<td>36\pm 1</td>
<td>57\pm 4†</td>
<td>26\pm 5*</td>
<td>44\pm 4†</td>
<td>24\pm 6*</td>
<td>46\pm 3†</td>
</tr>
<tr>
<td>LAD SWT (%)</td>
<td>25\pm 5</td>
<td>25\pm 4†</td>
<td>15\pm 1*</td>
<td>26\pm 6†</td>
<td>13\pm 4*</td>
<td>24\pm 4†</td>
</tr>
<tr>
<td>LCx SWT (%)</td>
<td>29\pm 7</td>
<td>26\pm 6</td>
<td>25\pm 5</td>
<td>28\pm 7</td>
<td>31\pm 6</td>
<td>28\pm 6</td>
</tr>
</tbody>
</table>

CON indicates control; G10, glybenclamide 10 \(\mu g \cdot kg^{-1} \cdot min^{-1}\) IC; G50, glybenclamide 50 \(\mu g \cdot kg^{-1} \cdot min^{-1}\) IC; LAD SWT, systolic wall thickening in the area perfused by the left anterior descending coronary artery; LCx SWT, systolic wall thickening in the control area perfused by the left circumflex coronary artery; NP, nitroprusside 1.5 \(\mu g \cdot kg^{-1} \cdot min^{-1}\) IC. Values are mean\pm SEM.

**Variable decreased in all three animals in response to glybenclamide.

†Variable increased in all three animals in response to nitroprusside.
duced coronary vasoconstriction resulted in ischemia-induced contractile dysfunction.

Despite the decrease in resting coronary blood flow, the increments in flow and contractile function produced by exercise were not attenuated by blockade of the K\textsuperscript{+}ATP channels. This suggests that K\textsuperscript{+}ATP channels maintain a basal level of activity that inhibits coronary tone during resting conditions but that they are not essential for coronary vasodilation that occurs in response to the increased myocardial oxygen demands produced by exercise. It is possible, however, that as glybenclamide inhibits activation of K\textsuperscript{+}ATP, other vasodilator systems are activated in response to further deterioration of the myocardial oxygen supply-demand balance with exercise. For example, Samaha and colleagues\textsuperscript{11} demonstrated that adenosine released from cardiac myocytes can attenuate the coronary vasoconstrictor effect of glybenclamide in the open-chest dog. There is evidence that adenosine and glybenclamide cause mutually competitive inhibition of each other's vascular actions.\textsuperscript{10,17} We have shown earlier that blockade of adenosine receptors does not affect exercise-induced coronary vasodilation in the absence of ischemia\textsuperscript{4} but does contribute to coronary vasodilation when exercise in the presence of a coronary artery stenosis results in myocardial ischemia.\textsuperscript{21} After administration of glybenclamide in the present study, ischemic myocardial hypofunction was present at rest and during each level of exercise, suggesting that increased adenosine production was likely. Such a role for adenosine is supported by the observation that in the normal coronary circulation, vasoconstriction by neurometopeptide Y leads to the release of endogenous adenosine, which attenuates the vasoconstriction.\textsuperscript{22} Whether adenosine and/or other vasodilator systems contributed to the exercise-mediated coronary vasodilation in the presence of K\textsuperscript{+}ATP channel blockade remains to be determined.

The mechanism by which the activity of coronary K\textsuperscript{+}ATP channels is regulated in the intact animal is incompletely understood. It has been suggested that physiological concentrations of ATP cause the channels to become inactive, whereas under conditions of decreased ATP the channels become activated.\textsuperscript{23,24} This concept appears to be supported by the finding that K\textsuperscript{+}ATP channels contributed to coronary vasodilation during global hypoxia in isolated guinea pig hearts\textsuperscript{10} and during myocardial ischemia distal to a coronary artery stenosis in an situ dog hearts.\textsuperscript{12} Similarly, the decreased coronary reactive hyperemic responses in both perfused hearts\textsuperscript{17} and intact animals (present study) appear to be in agreement with the concept that K\textsuperscript{+}ATP channels are activated under conditions of impaired ATP synthesis. A problem with this interpretation is the very low concentration of ATP required to cause K\textsuperscript{+}ATP channels to open in vitro studies. Thus, studies using the inside-out membrane patch-clamp technique have shown that ATP concentrations of no more than 1 mM inactivated more than 95% of the channels in cardiac muscle\textsuperscript{25} or arterial smooth muscle.\textsuperscript{26} In the intact cell, cytosolic ATP concentrations are normally in the millimolar range, suggesting that the K\textsuperscript{+}ATP channels would be inactive under physiological conditions. Despite these in vitro findings that suggest that K\textsuperscript{+}ATP channels would contribute minimally to coronary vasomotor tone during basal conditions, in the present study glybenclamide caused significant reductions of coronary blood flow in the resting intact animal.

Activation of K\textsuperscript{+}ATP channels that occurs in response to brief coronary artery occlusions also cannot be explained by a decrease in cytosolic ATP concentrations. Thus, using \textsuperscript{31}P nuclear magnetic resonance spectroscopy to measure beat-to-beat changes in high-energy phosphate content, Schwartz and colleagues\textsuperscript{26} found that myocardial ATP levels did not change during coronary artery occlusions up to 24 seconds in duration. To achieve myocardial ATP levels as low as 1 mM (20% of normal) requires at least 40 minutes of total coronary artery occlusion.\textsuperscript{27} Several explanations could account for the discrepancies between the in vitro inside-out patch-clamp observations and the present in vivo observations. One possible explanation involves cytosolic compartmentation of ATP; it is possible that changes in local levels of ATP could occur at the cell membrane (and thus in the vicinity of the K\textsuperscript{+}ATP channels) without a measurable change in overall cytosolic ATP levels. There is evidence to support a relation between local membrane-bound glycolytic ATP production and ion transport across the cell membrane in cardiac myocytes.\textsuperscript{28,29} In addition to changes in ATP, ADP levels may participate in regulation of K\textsuperscript{+}ATP channels by shifting the inactivation dose-response curve of ATP to higher concentrations.\textsuperscript{30} These and other as-yet-unknown mechanisms may be involved in activation of K\textsuperscript{+}ATP channels during resting conditions and during brief periods of ischemia in the intact heart.

Conclusions

Blockade of K\textsuperscript{+}ATP channels decreased resting coronary blood flow and attenuated coronary reactive hyperemia. However, exercise-induced increases in coronary blood flow were not prevented. These data demonstrate that activity of these channels contributes to maintenance of coronary blood flow under resting conditions and facilitates coronary vasodilation in response to a brief ischemic stimulus. However, further opening of K\textsuperscript{+}ATP channels is not essential for the coronary vasodilation that occurs during exercise.

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References

Role of K+ATP channels in coronary vasodilation during exercise.
D J Duncker, N S Van Zon, J D Altman, T J Pavek and R J Bache

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