ATP-Dependent K^+ Channels Contribute to Local Metabolic Coronary Vasodilation in Experimental Diabetes

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This study tested whether ATP-dependent K^+ channels (K_{ATP} channels) are an important mechanism of functional coronary hyperemia in conscious, instrument-implanted diabetic dogs. Data were collected at rest and during exercise before and after induction of diabetes with alloxan monohydrate (40–60 mg/kg intravenously). K_{ATP} channels were inhibited with glibenclamide (1 mg/kg intravenously). In nondiabetic dogs, arterial plasma glucose concentration increased from 4.8 ± 0.3 to 21.5 ± 2.2 mmol/l 1 week after alloxan injection. In nondiabetic dogs, exercise increased myocardial oxygen consumption (MVo_2) 3.4-fold, myocardial O_2 delivery 3.0-fold, and heart rate 2.4-fold. Coronary venous Po_2 decreased from 19.9 ± 0.8 mmHg at rest to 14.8 ± 0.8 mmHg during exercise. Diabetes significantly reduced myocardial O_2 delivery and lowered coronary venous Po_2 from 16.3 ± 0.6 mmHg at rest to 13.1 ± 0.9 mmHg during exercise. Glibenclamide did not alter the slope of the coronary venous Po_2 versus MVo_2 relationship in nondiabetic dogs. In diabetic dogs, however, glibenclamide further reduced myocardial O_2 delivery; coronary venous Po_2 fell to 9.0 ± 1.0 mmHg during exercise, and the slope of the coronary venous PO_2 versus MVo_2 relationship steepened. These findings indicate that K_{ATP} channels contribute to local metabolic coronary vasodilation in alloxan-induced diabetic dogs. *Diabetes* 51:1201–1207, 2002

Numerous investigations have focused on the role of ATP-dependent K^+ (K_{ATP}) channels in coronary blood flow regulation (1–14). These studies indicate that K_{ATP} channels are important in regulating coronary vascular resistance under baseline conditions (3–10), during hypoxic coronary vasodilation (11,12), and during reactive coronary hyperemia (1,4,13). However, it does not appear that K_{ATP} channels are required to increase coronary blood flow when myocardial metabolism is increased (3–5,7,8).

Recently, Kersten et al. (14) found that diabetes enhanced K_{ATP} channel–mediated coronary vasodilation of coronary arterioles, suggesting that K_{ATP} channels are important in local metabolic coronary vasodilation in diabetes. Supporting this notion are the results of Shimoni et al. (15) showing that the half-maximal inhibitory concentration (IC_{50}) for ATP-dependent inhibition of K_{ATP} channels was approximately twofold higher for channels from diabetic rat hearts. If there is an increase of K_{ATP} channel activity in diabetes, then oral hypoglycemic agents such as glibenclamide (Glyburide or Diabeta), which are K_{ATP} channel antagonists, could seriously impair control of coronary blood flow, especially when myocardial oxygen demand is elevated. Despite this fact, no study has examined whether K_{ATP} channels contribute to metabolic coronary vasodilation in an intact diabetic model. Furthermore, characterizing mechanisms that regulate coronary vascular tone is particularly important since coronary flow reserve (16–19), functional coronary hyperemia (18), and the balance between coronary blood flow and myocardial metabolism (20) are impaired in diabetic subjects.

Accordingly, this study was designed to determine whether K_{ATP} channels contribute to local metabolic coronary vasodilation in diabetic subjects. Experiments were conducted at rest and during graded treadmill exercise, with and without K_{ATP} channel blockade (glibenclamide, 1 mg/kg i.v.) (7,8), in chronically instrument-implanted dogs before and after induction of diabetes with alloxan monohydrate (40–60 mg/kg i.v.) (21).

RESEARCH DESIGN AND METHODS

Surgical preparation. This investigation was approved by the Institutional Animal Care and Use Committee and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publ. no. 85-23, revised 1996). Experiments were performed on six adult mongrel dogs of either sex (weighing 25–30 kg) taught to run on a motorized treadmill. Preanesthesia (acepromazine 0.03 mg/kg intramuscularly) was administered 30 min before induction of anesthesia with thiopental sodium (5 mg/kg i.v.). After endotracheal intubation, a surgical plane of anesthesia was maintained by mechanical ventilation with 1–3% isoflurane gas with supplemental oxygen. Using sterile technique, a left lateral thoracotomy was performed in the fifth intercostal space. A custom-made, coextruded polyurethane catheter (Putnam Plastics, Dayville, CT) was implanted in the descending thoracic aorta to measure aortic blood pressure and obtain arterial blood samples (8,22). A second polyurethane catheter was placed in the coronary sinus via a purse-string suture in the right atrial appendage for coronary venous blood sampling. The circumflex coronary artery was dissected free, and a flow transducer was placed around the artery. To avoid ventricular tissue injury, no devices were implanted in the myocardium and no surgical stitches were placed in the ventricles. A chest tube was placed to evacuate the pneumothorax, and the chest was closed in layers. The catheters and the flow transducer wire were tunneled subcutaneously and exteriorized between the scapulae. The incision was infiltrated with 2.5% bupivacaine, and

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K_{ATP} channels, ATP-dependent K^+ channels; MVo_2, myocardial oxygen consumption.

buprenorphine (Buprenex) (0.3 mg intramuscularly) was administered to minimize postoperative pain. Amoxicillin (Clavamox; 6.25 mg/lb) and aspirin (81 mg) were administered twice a day for 10 days after surgery. A nylon jacket (Alice King Chatham, Hawthorne, CA) was placed on the animals to protect the catheters and the flow transducer wire. A elastomeric balloon pump (Access Technologies, Skokie, IL) was connected to the coronary sinus catheter so that heparinized saline (5 units/ml) could be continuously infused at 2 ml/h. The aortic catheter was flushed daily and filled with heparinized saline (5,000 units/ml). The animals were allowed at least 7 days for recovery before the experiments were conducted.

Pressure and flow measurements. A coextruded polyurethane catheter was implanted in the aorta so that a high-fidelity catheter-tipped pressure transducer (Mikro-tip 3F; Millar Instruments, Houston, TX) could be inserted through it at the time of the experiment to measure aortic blood pressure (23). This pressure transducer was introduced into the polyurethane aortic catheter through a hemostatic control valve (Tuohy-Borst; Mallinckrodt Medical, Hazelwood, MO), which allowed arterial blood samples to be drawn while maintaining a fluid-tight seal around the catheter.

Phasic and mean coronary blood flow were continuously measured throughout the experimental protocol with an ultrasonic, perivascular flow transducer (Transonic Systems, Ithaca, NY). After experiments were completed, the animals were killed with sodium pentobarbital, and the circumflex perfusion territory was dyed with Evans blue. The weight of the dyed tissue was used to calculate coronary blood flow per gram of perfused myocardium.

Blood sampling. Arterial and coronary venous blood samples were collected simultaneously in heparinized syringes that were immediately sealed and placed on ice. The samples were analyzed in duplicate for pH, P_{CO_2}, P_{O_2}, hematocrit, and oxygen content with an automatic blood gas analyzer (Synthesis 30) and CO-oximeter (model 682) system (Instrumentation Laboratories, Lexington, MA). Plasma glucose and lactate concentrations were measured with a Radiometer analyzer (model EML105; Radiometer, Bronshoj, Denmark). Myocardial oxygen consumption (MV_{O_2}) ($\mu l\ O_2 \cdot min^{-1} \cdot g^{-1}$) was calculated by multiplying coronary blood flow per gram of perfused tissue by the coronary arteriovenous difference in oxygen content. Lactate uptake ($\mu mol \cdot min^{-1} \cdot g^{-1}$) was calculated by multiplying coronary plasma flow per gram of perfused tissue by the coronary arteriovenous difference in plasma lactate concentration.

Experimental protocol. The hypothesis that K_{ATP} channels are an important mechanism of functional coronary hyperemia in diabetes was tested at rest and during graded treadmill exercise with and without the K_{ATP} channel inhibitor glibenclamide. Each animal served as its own control. The dose of glibenclamide (1 mg/kg i.v.) used in this investigation has been used in previous studies (7,8,24) and was found to effectively block the vasodilating action of the K_{ATP} channel opener cromakalim (25). After an overnight fast, coronary blood flow, aortic pressure, and heart rate were continuously measured while the dogs were resting in a sling and then during three levels of treadmill exercise: *1*) 2 mph, 0% grade; *2*) 3 mph, 5% grade; and *3*) 4 mph, 10% grade. The animals exercised at similar levels (i.e., speed and percent grade) before and after alloxan treatment. Arterial and coronary venous blood samples were collected when hemodynamic variables were stable at each exercise level. Each exercise period was ~2 min in duration, and the animals were allowed to rest sufficiently between each level for hemodynamic variables to return to baseline.

After nondiabetic control experiments were conducted, alloxan monohydrate (40–60 mg/kg) was administered intravenously over 1 min to induce diabetes (21). Alloxan was prepared as a 5% solution in citrate buffer (pH 4.0–4.5). The dogs were fasted at least 12 h before the injection of alloxan and were then fed immediately. A dog was considered diabetic if its plasma glucose concentration was >11 mmol/l. The experimental protocol described above was repeated in the diabetic animals at least 7 days after the alloxan injection with and without glibenclamide. In addition, three experiments were also conducted at rest and during exercise with glibenclamide in nondiabetic dogs to confirm the previous findings that K_{ATP} channels are not required for exercise-induced coronary vasodilation (3–5,7,8).

Assessing the relationship between coronary blood flow and myocardial metabolism. Changes in myocardial oxygen supply must be evaluated in relation to MV_{O_2}, the primary determinant of coronary blood flow. Thus, the present data were analyzed by plotting changes in myocardial oxygen delivery and coronary venous P_{O_2} directly with changes in MV_{O_2}. Coronary venous P_{O_2} is a sensitive index of tissue oxygenation, and the plot of coronary venous P_{O_2} versus MV_{O_2} is a sensitive way to determine whether the relationship between coronary blood flow and myocardial metabolism has been altered. On this plot, a vasodilator influence will either shift the regression line upward or make the slope less negative. A vasoconstrictor influence will shift the regression line downward or make the slope more negative. Influences on baseline flow will shift the relationship in a parallel manner, whereas an intervention that affects only exercise vasodilation will change the slope. Importantly, by plotting coronary response variables as a function of MV_{O_2}, these plots account for any drug-induced changes in heart rate, blood pressure, or contractility that may significantly affect myocardial oxygen demand. Many studies have used this approach to analyze mechanisms of coronary blood flow control (3–5,8,20,22,24).

Statistical analyses. Data are presented as means ± SE. Hemodynamic variables were recorded with a Hewlett Packard 7758A recorder and analyzed with a Sonometrics Sonolab 3.1.4 data acquisition system. Statistical testing was directed to detect overall treatment effects, i.e., diabetes-control versus diabetes-glibenclamide. A repeated-measures ANOVA was used to test for differences between coronary and systemic hemodynamic variables at rest and during exercise. When significance was found with ANOVA ($P < 0.05$), a Student-Newman-Keul's multiple comparison test was performed. Linear regression analysis was used to compare the slopes of response variables (oxygen delivery and coronary venous P_{O_2}) plotted versus MV_{O_2}.

RESULTS

Hemodynamic, blood gas, and metabolic variables at rest and during exercise are given in Table 1. Average blood urea nitrogen and creatinine levels after 1 week of alloxan treatment were 34.5 ± 11.9 and 1.75 ± 0.60 mg/dl, respectively. (Reference values for dogs range from 7 to 27 mg/dl for blood urea nitrogen and from 0.5 to 1.8 mg/dl for creatinine.) Body weight was reduced from 28.7 ± 3 to 27.5 ± 3 kg after alloxan treatment. Average arterial plasma glucose concentration increased from 4.8 ± 0.3 mmol/l in nondiabetic control dogs to 21.5 ± 2.2 mmol/l ~1 week after alloxan injection. Glibenclamide did not significantly alter the arterial plasma glucose concentration in the alloxan-treated dogs. In the nondiabetic control dogs, exercise significantly increased MV_{O_2} 3.4-fold, myocardial oxygen delivery 3.0-fold, and heart rate 2.4-fold (Table 1). Coronary venous P_{O_2} decreased from 19.9 ± 0.8 mmHg at rest to 14.8 ± 0.8 mmHg at the highest level of exercise. Diabetes significantly reduced the exercise-induced increase in myocardial oxygen delivery to 2.5-fold and lowered the coronary venous P_{O_2} to 16.3 ± 0.8 mmHg at rest and to 13.3 ± 0.8 mmHg during exercise.

Blockade of K_{ATP} channels with glibenclamide in the diabetic dogs tended to lower coronary blood flow (Fig. 1*A*) and MV_{O_2} (Fig. 1*B*) at rest and during exercise. K_{ATP} channel blockade decreased myocardial oxygen delivery at the highest level of exercise (Fig. 1*C*) and significantly reduced coronary venous P_{O_2} to 14.3 ± 0.7 mmHg at rest and to 9.4 ± 0.9 mmHg at the highest level of exercise (Fig. 1*D*).

The relationship between myocardial oxygen delivery and MV_{O_2} is shown in Fig. 2. Glibenclamide treatment significantly decreased the slope of this relationship ($P = 0.03$), indicating that K_{ATP} channels are important to functional exercise coronary hyperemia in alloxan-induced diabetes. Because of the normally high oxygen extraction by the left ventricle (8,22,24), the data points and regression lines lie extremely close to the line of 100% oxygen extraction (oxygen consumed = oxygen delivery) illustrated in Fig. 2. Thus, significant differences in oxygen delivery at a given MV_{O_2} are hard to visualize. The difference between these data points and the line of 100% oxygen extraction represents the oxygen extraction reserve of the myocardium, which is shown in Fig. 3. Glibenclamide significantly reduced the percent myocardial oxygen reserve at rest and at each level of exercise, indicating that K_{ATP} channel blockade impaired the balance between oxygen delivery and consumption and

TABLE 1
Hemodynamic, blood gas, and metabolic variables at rest and during graded treadmill exercise

	n	Rest	Exercise		
			Level 1	Level 2	Level 3
Coronary blood flow (ml · min^{-1} · g^{-1})					
Control	6	0.59 ± 0.04	1.01 ± 0.05	1.25 ± 0.09	1.65 ± 0.16
Diabetes	6	0.55 ± 0.06	0.69 ± 0.09*	0.94 ± 0.14*	1.19 ± 0.17*
Diabetes + glibenclamide	6	0.48 ± 0.03	0.65 ± 0.06*	0.83 ± 0.06*	1.05 ± 0.11*
Myocardial oxygen delivery (μl O_2 · min^{-1} · g^{-1})					
Control	6	105 ± 6	198 ± 9	239 ± 14	316 ± 27
Diabetes	6	94 ± 10	130 ± 19*	178 ± 27*	236 ± 35*
Diabetes + glibenclamide	6	80 ± 7	116 ± 14*	144 ± 14*	193 ± 25*†
Myocardial O_2 consumption (μl O_2 · min^{-1} · g^{-1})					
Control	6	80 ± 4	169 ± 7	205 ± 11	272 ± 22
Diabetes	6	78 ± 7	114 ± 18*	156 ± 24	211 ± 34*
Diabetes + glibenclamide	6	69 ± 6	106 ± 13*	134 ± 14*	180 ± 26*
Mean aortic pressure (mmHg)					
Control	6	106 ± 4	114 ± 5	112 ± 7	119 ± 4
Diabetes	6	105 ± 5	110 ± 5	113 ± 2	116 ± 3
Diabetes + glibenclamide	6	115 ± 6	120 ± 5	122 ± 3	123 ± 3
Heart rate (beats/min)					
Control	6	98 ± 7	155 ± 11	183 ± 9	236 ± 19
Diabetes		95 ± 13	134 ± 13	172 ± 10	206 ± 8
Diabetes + glibenclamide	6	81 ± 12*	114 ± 9*†	153 ± 16*†	187 ± 18*†
Arterial pH					
Control	6	7.42 ± 0.01	7.44 ± 0.01	7.46 ± 0.02	7.45 ± 0.02
Diabetes	6	7.42 ± 0.02	7.43 ± 0.03	7.42 ± 0.02	7.43 ± 0.02
Diabetes + glibenclamide	6	7.40 ± 0.02	7.43 ± 0.02	7.43 ± 0.03	7.44 ± 0.02
Coronary venous pH					
Control	6	7.38 ± 0.01	7.41 ± 0.02	7.40 ± 0.02	7.39 ± 0.02
Diabetes	6	7.37 ± 0.02	7.39 ± 0.03	7.39 ± 0.02	7.39 ± 0.03
Diabetes + glibenclamide	6	7.37 ± 0.02	7.39 ± 0.02	7.40 ± 0.02	7.39 ± 0.02
Arterial P_{O_2} (mmHg)					
Control	6	87 ± 2	99 ± 4	94 ± 3	84 ± 4
Diabetes	6	86 ± 2	93 ± 3	88 ± 4	86 ± 3
Diabetes + glibenclamide	6	87 ± 3	89 ± 3*	90 ± 4	85 ± 4
Coronary venous P_{O_2} (mmHg)					
Control	6	19.9 ± 0.8	15.1 ± 0.8	15.0 ± 0.6	14.8 ± 0.8
Diabetes	6	16.3 ± 0.8	13.7 ± 0.8	13.3 ± 0.7	13.3 ± 0.8
Diabetes + glibenclamide	6	14.3 ± 0.7*†	10.6 ± 0.7*†	9.4 ± 0.9*†	9.4 ± 0.9*†
Arterial P_{CO_2} (mmHg)					
Control	6	34 ± 1	28 ± 2	28 ± 1	28 ± 2
Diabetes	6	30 ± 1	27 ± 2	27 ± 1	26 ± 2
Diabetes + glibenclamide	6	30 ± 2	28 ± 2	27 ± 2	27 ± 2
Coronary venous P_{CO_2} (mmHg)					
Control	6	47 ± 2	44 ± 2	43 ± 2	43 ± 2
Diabetes	6	45 ± 3	42 ± 3	41 ± 2	40 ± 2
Diabetes + glibenclamide	6	44 ± 3	42 ± 2	41 ± 2	42 ± 2
Hematocrit (%)					
Control	6	38 ± 2	40 ± 2	40 ± 2	42 ± 3
Diabetes	6	40 ± 1	40 ± 2	41 ± 1	42 ± 3
Diabetes + glibenclamide	6	36 ± 1	39 ± 2	39 ± 2	41 ± 2
Arterial oxygen content (ml O_2/dl blood)					
Control	6	17.9 ± 0.7	19.7 ± 0.9	19.5 ± 0.9	19.7 ± 1.1
Diabetes	6	17.8 ± 0.6	19.1 ± 0.9	18.6 ± 0.4	19.1 ± 0.8
Diabetes + glibenclamide	6	16.8 ± 0.6	17.7 ± 0.9	17.1 ± 0.6	18.2 ± 0.8
Coronary venous oxygen content (ml O_2/dl blood)					
Control	6	4.3 ± 0.3	2.8 ± 0.2	2.7 ± 0.2	2.7 ± 0.2
Diabetes	6	2.8 ± 0.2	2.5 ± 0.3	2.3 ± 0.3	2.3 ± 0.4
Diabetes + glibenclamide		2.3 ± 0.2*	1.5 ± 0.2*†	1.3 ± 0.2*†	1.3 ± 0.3*†
Arterial plasma glucose concentration (mmol/l)					
Control	6	4.8 ± 0.3	4.6 ± 0.2	4.6 ± 0.3	5.2 ± 0.3
Diabetes	6	21.5 ± 2.2*	20.5 ± 2.4*	21.3 ± 2.3*	21.8 ± 1.9*
Diabetes + glibenclamide	6	19.9 ± 2.5*	20.1 ± 2.5*	19.5 ± 2.5*	20.8 ± 2.7*
Myocardial lactate uptake (μmol · min^{-1} · g^{-1})					
Control	6	0.08 ± 0.03	0.21 ± 0.08	0.42 ± 0.14	0.77 ± 0.32
Diabetes	6	0.01 ± 0.01	0.03 ± 0.02	0.06 ± 0.03	–0.04 ± 0.04*
Diabetes + glibenclamide	6	0.01 ± 0.01	0.06 ± 0.02	0.08 ± 0.02	0.05 ± 0.05*

Data are means ± SE. *$P < 0.05$ vs. diabetes, same condition; †$P < 0.05$ vs. control, same condition.

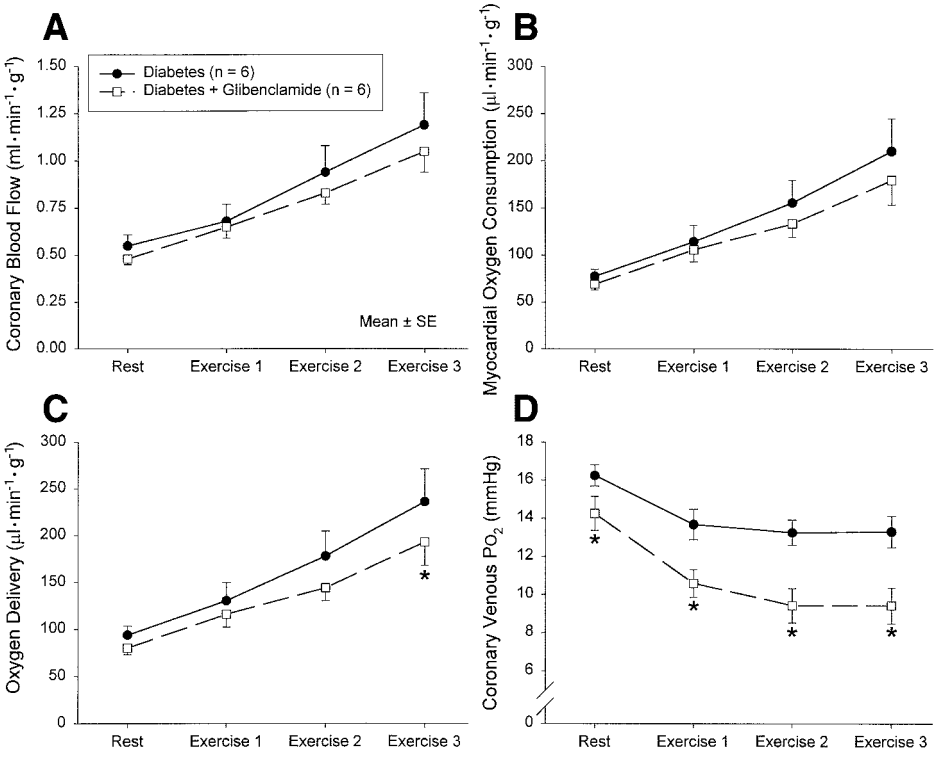

FIG. 1. Average results of coronary blood flow (*A*), MVo_2 (*B*), oxygen delivery (*C*), and coronary venous Po_2 (*D*) at rest and during exercise in diabetic dogs with and without glibenclamide treatment. Blockade of K_{ATP} channels tended to lower coronary blood flow and MVo_2 at rest and during exercise. Glibenclamide significantly reduced oxygen delivery at the highest level of exercise and reduced coronary venous Po_2 at each condition. *P < 0.05 vs. diabetes, same condition.

forced the heart to use more of its limited oxygen extraction reserve.

A more sensitive index of the relationship between coronary blood flow and myocardial metabolism is shown in the plot of coronary venous Po_2 versus MVo_2 (Fig. 4) (4,5,8,20,22,24). K_{ATP} channel blockade with glibenclamide made the slope of this relationship more negative (P = 0.01), indicating that K_{ATP} channels contribute to local metabolic coronary vasodilation in experimental diabetes.

Additional experiments (n = 3) were also conducted to confirm earlier findings of the effects of K_{ATP} channel blockade on coronary blood flow control during exercise. Results from these experiments, plus a replotting of the data from Richmond et al. (8), are shown in Fig. 5. Consistent with previous studies (3,8), K_{ATP} channel blockade with glibenclamide in the normal (control) dogs resulted in a significant parallel shift downward in the relationship between coronary venous Po_2 and MVo_2 (P < 0.0001). However, the slope of this relationship did not become more negative (P = 0.66), indicating that K_{ATP} channels are not required for exercise-induced coronary vasodilation in normal, nondiabetic dogs.

DISCUSSION

The present study is the first to examine whether K_{ATP} channels contribute to local metabolic coronary vasodila-

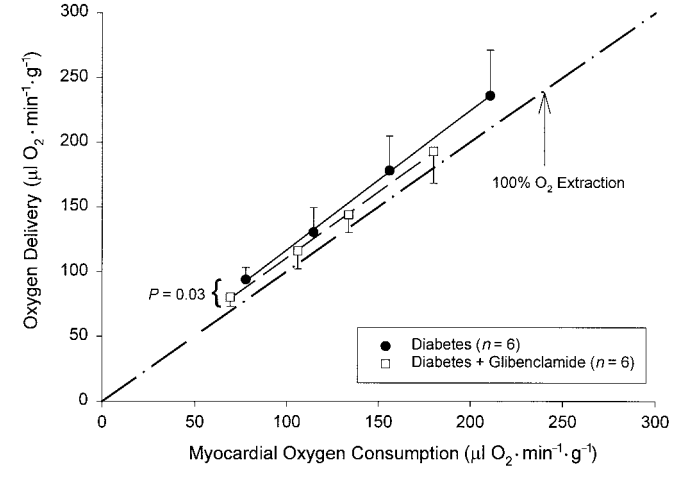

FIG. 2. The relationship between myocardial oxygen delivery and MVo_2 in diabetic dogs with and without glibenclamide treatment. Glibenclamide significantly decreased the slope of this relationship, indicating that K_{ATP} channels contribute to exercise-induced coronary vasodilation in alloxan-induced diabetic dogs. The point at which the oxygen delivery equals the oxygen consumed (i.e., 100% extraction) is represented by the dash-dot-dash line.

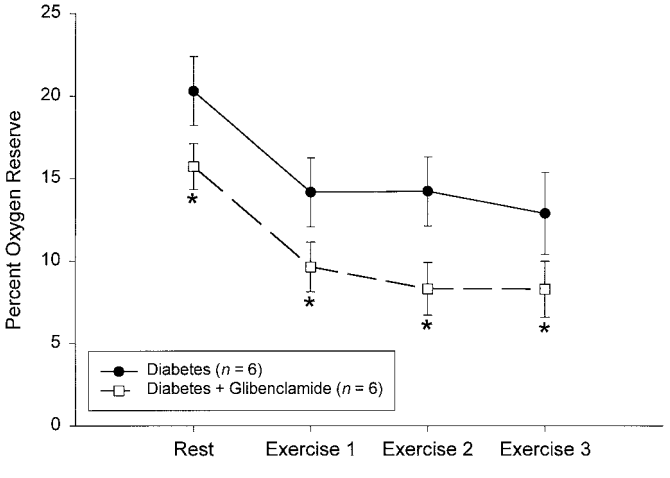

FIG. 3. Average percent oxygen reserve in diabetic dogs with and without glibenclamide treatment. Glibenclamide significantly reduced the myocardial oxygen reserve at rest and during exercise, indicating that K_{ATP} channel blockade impaired the balance between coronary blood flow and myocardial metabolism, which forced the heart to use its limited oxygen extraction reserve. *P < 0.05 vs. diabetes, same condition.

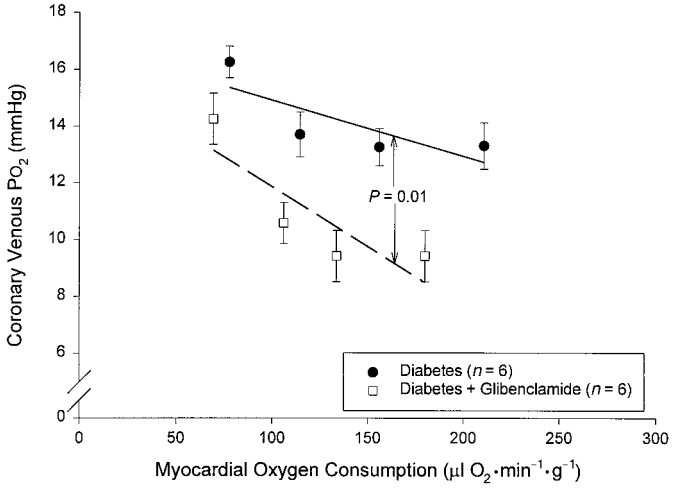

FIG. 4. Relationship between coronary venous P_{O_2} and MV_{O_2} in diabetic dogs with and without glibenclamide treatment. Glibenclamide made the slope of this relationship more negative, indicating that K_{ATP} channels contribute to local metabolic coronary vasodilation in alloxan-induced diabetic dogs.

tion during exercise in experimental diabetes. Relative to diabetes alone, K_{ATP} channel blockade with glibenclamide significantly reduced myocardial oxygen delivery at the highest level of exercise (Fig. 1*C*) and reduced the slope of the relationship between myocardial oxygen delivery and MV_{O_2} (Fig. 2), thereby decreasing the oxygen reserve of the myocardium (Fig. 3). In addition, glibenclamide made the slope of the relationship between coronary venous P_{O_2} and MV_{O_2} more negative (Fig. 4). These findings indicate that K_{ATP} channels are an important mechanism of functional coronary hyperemia in experimental diabetes.

K_{ATP} channels, coronary blood flow control, and diabetes. Whether K_{ATP} channels contribute to functional coronary hyperemia in diabetic subjects has not been previously investigated. This hypothesis was examined in the present study by relating changes in myocardial oxygen delivery and coronary venous P_{O_2} directly with changes in MV_{O_2}. Thus, changes in myocardial oxygen supply are related directly to changes in myocardial oxygen demand. When K_{ATP} channels were inhibited in the diabetic dogs with glibenclamide, the slope of the coronary venous P_{O_2} vs. MV_{O_2} relationship (Fig. 4) was steepened, i.e., became more negative. This finding indicates that K_{ATP} channels normally contribute to local metabolic coronary vasodilation in the diabetic animals. In addition, glibenclamide significantly decreased the slope of the relationship between myocardial oxygen delivery and MV_{O_2} (Fig. 2). In other words, the oxygen delivery to the myocardium was impaired by glibenclamide as MV_{O_2} was increased during exercise. It should be appreciated that the slope of this relationship can only decrease to a limited extent, since the myocardial oxygen extraction is so high at rest (>80% in the diabetic group). Because K_{ATP} channel blockade limited the delivery of oxygen to the myocardium, the heart was forced to use more of its limited extraction reserve to meet the metabolic requirements of the tissue. This fact is also evidenced by the reduction in the oxygen reserve of the myocardium (Fig. 3).

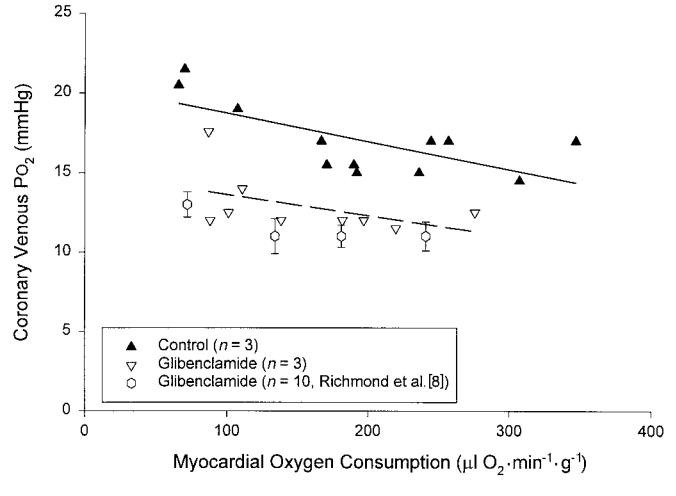

FIG. 5. Relationship between coronary venous P_{O_2} and MV_{O_2} in nondiabetic (control) dogs with and without glibenclamide treatment. Glibenclamide did not alter the slope but did produce a significant parallel shift downward in this relationship, indicating that K_{ATP} channels are not required for local metabolic coronary vasodilation in normal (nondiabetic) dogs. In addition, a replotting of the average data from Richmond et al. (8) demonstrates that our results are consistent with earlier studies.

The present findings that K_{ATP} channels contribute to local metabolic coronary vasodilation in alloxan-induced diabetic dogs are supported by the earlier findings of Kersten et al. (14), who found that the coronary vasodilatory response of coronary arterioles (<100 μm) to the K_{ATP} channel agonist aprikalim was significantly enhanced in alloxan-induced diabetic dogs relative to euglycemic (control) dogs. The coronary arteriolar response to aprikalim was also enhanced in nondiabetic hyperglycemic dogs, suggesting that high circulating glucose levels may be responsible for the increase in K_{ATP} channel activity. The results of Shimoni et al. (15), showing that the IC_{50} for ATP-dependent inhibition of K_{ATP} channels was approximately twofold higher for channels from diabetic rat hearts, also support the present findings. Further studies are needed to determine whether hyperglycemia per se is responsible for the apparent increase in K_{ATP} channel activity detected in this investigation.

It should be acknowledged that glibenclamide also has nonspecific effects on other coronary vasodilator mechanisms that may have contributed to the present findings. Most notably, glibenclamide also significantly attenuates adenosine-mediated coronary vasodilation (2,4,5,11,13,24, 25). Adenosine does not contribute to local metabolic coronary vasodilation during exercise in nondiabetic animals (22), and furthermore, adenosine-induced coronary vasodilation is attenuated in diabetic animals (21,26–28). Therefore, it is unlikely that glibenclamide-induced attenuation of adenosine vasodilation was directly responsible for the findings of this study. However, our laboratory recently reported that the balance between coronary blood flow and myocardial metabolism is significantly impaired at rest and during exercise in alloxan-induced diabetic dogs (20). Data from the present study support this finding (Table 1). This imbalance could lead to a compensatory increase in cardiac adenosine release, in which case the present treatment with glibenclamide would have blunted vasodilation of both K_{ATP} channels and adenosine. Either way, this study has demonstrated that sulfonylurea treatment with

glibenclamide significantly attenuates functional coronary hyperemia in diabetic dogs.

Previous investigations have examined the role of K_{ATP} channels in modulating coronary vascular resistance in nondiabetic animals. The consensus of these studies is that K_{ATP} channels contribute to control of resting coronary blood flow (3–10) but are not required for coronary hyperemia during increases in MVO_2 (3–5,7,8). Data from control (nondiabetic) animals studied in this investigation support these conclusions. At rest, glibenclamide decreased resting coronary flow and venous PO_2 (Fig. 5). During exercise, glibenclamide did not compromise coronary vasodilation in these animals, since the K_{ATP} channel blockade resulted in a parallel shift of the coronary venous PO_2 versus MVO_2 relationship but did not significantly alter the slope (Fig. 5).

Alloxan-induced diabetes. The present experiments were conducted in alloxan-induced diabetic animals whose fasting plasma glucose concentration averaged ~20 mmol/l (360 mg/dl). Acute glibenclamide administration did not significantly reduce the arterial plasma glucose concentration (Table 1), indicating that an increase in the release of insulin from the pancreas was not a confounding effect of glibenclamide treatment in this study. This effect is most likely due to the fact that alloxan destroys the islets of Langerhans in pancreas, and thus insulin release could not be significantly altered (29). The animals were not treated with insulin and thus represent a model of poorly controlled type 1 diabetes, i.e., chronic hypoinsulinemia and hyperglycemia. Whether K_{ATP} channels contribute to exercise-induced coronary vasodilation in type 2 (insulin-resistant) diabetes merits future investigation.

Clinical implications. Sulfonylurea-derivative drugs such as glibenclamide have been used for many years to increase insulin release in patients with non-insulin-dependent diabetes. However, studies showing that blockade of K_{ATP} channels increases coronary vascular resistance in experimental nondiabetic animals (3–10) has led to an unresolved debate as to whether sulfonylurea drugs should be used in patients with diabetes, especially those with ischemic heart disease (30,31). In earlier studies designed to examine the effect of diabetes on coronary flow reserve, some patients had received oral hypoglycemic therapy (18,32). Because the drug was withheld on the day of the investigation, its effect on coronary flow control was not determined. However, other investigations have reported a significant decrease in forearm vascular blood flow (33) and in calf reactive hyperemia (34) in nondiabetic humans after oral glibenclamide treatment.

It is also important to point out that K_{ATP} channels have different properties in different tissues. The IC_{50} for glibenclamide is ~4 nmol/l in pancreatic β-cells and ~27 nmol/l in cardiac myocytes (35,36). Its lower potency in cardiac myocytes suggests that glibenclamide could possibly affect pancreatic insulin release without influencing coronary vasomotor tone at clinically administered doses. However, the fact that oral glibenclamide reduced peripheral blood flow control in nondiabetic patients argues against this hypothesis (33,34). To date, no study has directly examined whether oral hypoglycemic drugs significantly impair coronary vasodilation in diabetic humans.

Conclusion. This study is the first to show that K_{ATP} channels contribute to local metabolic coronary vasodilation in experimental diabetic animals. This finding is important because it is well established that K_{ATP} channels are not required for functional coronary hyperemia in normal (nondiabetic) animals (3–5,7,8). Future clinical studies are needed to determine whether oral hypoglycemic therapy with agents such as glibenclamide compromises coronary vasodilation in patients with diabetes.

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