Coronary Vascular K⁺ATP Channels Contribute to the Maintenance of Myocardial Perfusion in Dogs With Pacing-Induced Heart Failure

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The functional role of coronary vascular ATP-sensitive potassium (K⁺ATP) channels in the regulation of coronary blood flow (CBF) has not been determined in chronic heart failure (CHF). To test the hypothesis that K⁺ATP channels contribute to myocardial perfusion in HF, we examined the effects of intracoronary infusion of glibenclamide, an inhibitor of K⁺ATP channels, on basal CBF in control and CHF dogs. CHF was produced in mongrel dogs by pacing the right ventricle for 4 weeks. Under anesthesia, CBF in the left anterior descending coronary artery, other hemodynamic and metabolic parameters, or regional myocardial blood flow were measured. Basal CBF was less in CHF dogs than in controls. Glibenclamide at the graded doses (5, 15 and 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) decreased CBF in both control and CHF dogs. The percentage decrease in CBF with glibenclamide at 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ was greater ($p<0.01$) in CHF dogs than in controls. The greater decrease in CBF with glibenclamide at 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ was associated with myocardial ischemia. Glibenclamide decreased myocardial blood flow in each sublayer of the myocardium in the 2 groups. These results suggest that the basal activity of coronary vascular K⁺ATP channels is increased in CHF dogs but not in controls. This may contribute to the maintenance of myocardial perfusion in CHF. (*Jpn Circ J* 2000; 64: 701–707)

Key Words: Coronary circulation; Heart failure; K⁺ATP channels

Since the discovery of adenosine 5'-triphosphate (ATP)-sensitive potassium channels,¹ their functional role in the cardiovascular system has been extensively investigated.^{2–4} In vivo evidence suggests that K⁺ATP channels contribute to the regulation of coronary blood flow (CBF); maintenance of basal CBF,^{5,6} as well as in ischemic/hypoxic,^{7–9} exercise-induced/metabolic^{10–13} or autoregulatory¹⁴ coronary vasodilation. These previous studies have demonstrated that cardiovascular K⁺ATP channels are indeed functionally activated during transient or short-term stimuli that are physiologically relevant. However, the role of K⁺ATP channels in the control of CBF during the chronic stress that occurs in the heart failure process has been poorly understood.

Chronic heart failure (CHF) is accompanied by increases in metabolic stress, probably because of tachycardia, cardiac enlargement, myocardial energy depletion, and neurohumoral activation. These changes may enhance the activity of cardiovascular K⁺ATP channels;^{2–4} activation of the K⁺ATP channels in these conditions may afford cardiovascular protection.⁴ Earlier studies in animal and human models of CHF have shown that CBF is impaired at rest and during physiological stimuli compared with controls,^{15–18} suggesting that coronary vascular tone is increased during CHF. It is possible that the enhanced coronary vascular tone (ie, myocardial hypoperfusion) in CHF is compen-

sated by the vasodilation induced by endogenous vasodilator systems such as the K⁺ATP channels. Data available from experimental models of CHF to date suggest that the activity of the K⁺ATP channels of cardiac myocytes is reduced.^{19,20} However, the activity of the K⁺ATP channels of the coronary artery and the functional role of the channels in the regulation of CBF has not been determined in CHF.

Thus, in the present study, we tested the hypothesis that the basal activity of the coronary vascular K⁺ATP channels is enhanced in CHF, which may contribute to the maintenance of myocardial perfusion in CHF. We used a dog model of pacing-induced CHF to investigate whether intracoronary infusion of glibenclamide, a specific inhibitor of K⁺ATP, decreases basal CBF to a greater extent and produces myocardial ischemia. We also investigated the effects of glibenclamide on regional myocardial blood flow at rest.

Methods

Induction of Pacing-Induced Heart Failure

This study was approved by the Committee on the Ethics of Animal Experiments, Faculty of Medicine, Kyushu University, and was conducted according to the Guidelines for Animal Experiments of the Faculty of Medicine, Kyushu University, and Law (No. 105) and Notification (No. 6) of the Japanese Government.

Experiments were performed in adult mongrel dogs (16–24 kg body weight). Under general anesthesia and fluoroscopic guidance, a bipolar pacing lead (1236T, Pace Setter Inc, Sylmer, CA, USA) was introduced into the right external jugular vein and advanced to the apical area of the right ventricle. The dogs were then allowed to recover from

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the surgery.

CHF was induced by rapid ventricular pacing at 240 beats/min for 4 weeks (CHF dogs) as described previously.²¹ In some dogs, the ventricular pacing was performed for only 1 week (Pacing dogs). Ventricular pacing was performed continuously by connecting the lead to an external pulse generator (Nihon-Kohden, Tokyo, Japan). Control dogs were treated in an identical manner to the CHF dogs, but without pacing.

On the day of the final study, all dogs underwent echocardiography studies in the conscious state in sinus rhythm after a 15-min stabilization period. Echocardiographic images were obtained with an ultrasonograph (SSH-65A, Toshiba Medical Inc, Tokyo, Japan). Left ventricular (LV) short-axis (cross-sectional) views were recorded at the papillary muscle level, and the internal left ventricular dimensions and the thickness of the lateral wall of the left ventricle were measured. The left ventricular ejection fraction (percent) was then calculated by the use of the formula $100 \times ([LV \text{ end-diastolic dimension} - LV \text{ end-systolic dimension}]^3 / LV \text{ end-diastolic dimension}^3)$.

Surgical Preparation of Measurements for the Final Experiment

After echocardiography, the following preparations were performed.^{10,14} The dogs were sedated with diazepam (1 mg), intubated after intravenous administration of pancuronium bromide (0.08 mg/kg), and ventilated with a respirator. They were then anesthetized with intravenous infusion of α -chloralose (3.75 mg/min) and urethane (37.5 mg/min). A thoracotomy was performed in the left fourth intercostal space, and the heart was suspended in a pericardial cradle. A heating pad was used to maintain the rectal temperature of animals within the range of 36–37.0°C.

A transit-time ultrasonic flow probe was placed at the mid-portion of the left anterior descending coronary artery (LAD).¹⁰ Heparin-filled miniature tubing (2F) was inserted into a small branch of the LAD immediately distal to the flow probe for drug infusions. A 3F catheter was inserted into the great cardiac vein and advanced into the anterior interventricular coronary vein for venous blood sampling. A 7F catheter was inserted into the aortic arch through the left carotid artery for measurement of aortic pressure (AoP), and a 7F catheter-tip pressure transducer was inserted into the left ventricular cavity through the left atrium for the measurement of left ventricular pressure (LVP). The pacing wire was attached to the left atrial appendage.

AoP was measured using a strain-gauge transducer (TP-400T, Nihon-Kohden). A cardiometer triggered by AoP pulses was used to monitor heart rate (HR). LVP was measured with a catheter-tip transducer (PC-350, Millar Instruments, Houston, TX, USA) and the positive first derivative of LVP (LV dp/dt) was obtained by electronic differentiation. CBF was measured with an ultrasonic flow meter (T201, Transonic System, Ithaca, NY, USA). All variables were continuously monitored and recorded using a polygraph system (RM-6000, Nihon-Kohden).

To determine the arterio-venous oxygen difference (AVOD), we measured the oxygen saturation of arterial and coronary venous blood.¹⁰ The oxygen saturation of paired blood samples from the aortic arch (SaO₂) and the coronary vein (SvO₂) was measured with a calibrated oxygen analyzer (Oxymeter type PWA-200S, ERMA, Tokyo, Japan). The hemoglobin content in venous blood samples was also measured. AVOD was calculated by the following formula:

$$AVOD(\text{ml/L}) = [1.36(\text{ml/g Hb}) \times \text{blood hemoglobin content}(\text{g/dl}) \times 10 \times \{\text{SaO}_2(\%) - \text{SvO}_2(\%)\} / 100].$$

The partial pressure of O₂ (PO₂) and carbon dioxide (PCO₂) and the pH in arterial and coronary venous blood were measured with a gas analyzer (model 238, Chiba Corning Inc, Tokyo, Japan). The arterial and coronary venous concentrations of lactate were also measured with a lactate analyzer (2,700 Select, Yellow Springs Instrument Inc). After the experiments, animals were killed with a lethal dose of α -chloralose, and the hearts were excised. After the LAD was occluded at the site of the flow probe, Evans blue dye was injected into the LAD to determine the myocardial area distal to the flow probe. CBF was then expressed in ml/min per unit of myocardial mass (100 g).

The whole blood cell count and serum parameters (C-reactive protein, blood urea nitrogen, creatinine, sodium, and potassium) were measured using the Total Blood Analyzer (Vision Analyzer, Abbott Laboratories).

Regional myocardial blood flow (MBF) was determined with a colored microsphere technique (Dye-Track, Triton Technologies Inc) as previously described.²² For each measurement, approximately 5 million microspheres with diameter of 15 μm suspended in 5 ml of saline containing 0.02% Tween 80 were injected through the catheter implanted in the left atrium. Each microsphere injection was followed by a 5-ml flush of saline. The withdrawal of arterial reference blood samples was started 15 s before injection of the microspheres and continued for 120 s at a rate of 10 ml/min through the catheter inserted into the descending aorta. Myocardial samples were obtained from the following 2 regions: the LV free wall perfused by LAD distal to the tube for drug infusion into which glibenclamide was infused, and LV free wall perfused by the left circumflex coronary artery without glibenclamide infusion. Each sample was subdivided into the subendocardial, mid-wall and subepicardial layers, weighed and then dissolved in 7 ml of 4 mol/L KOH solution containing 2% Tween 80 solution in a 10-ml glass tube. Regional MBF ($\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) was determined using a previously described technique.²³

Drugs

Glibenclamide, adenosine and sodium nitroprusside were obtained from Sigma (St Louis, MO, USA) and acetylcholine from Daiichi (Tokyo). Glibenclamide was dissolved in 4% glucose solution containing 0.01 mol/L NaOH. Other drugs were dissolved in normal saline.

Experimental Protocols

After completion of the surgical preparation, animals were studied when all hemodynamic parameters were stabilized. HR was maintained at approximately 160 beats/min by atrial pacing during the experiments.

Protocol 1 The effects of glibenclamide on basal CBF were determined in 10 control dogs, 10 Pacing dogs and 10 CHF dogs. Physiological saline vehicle at 1 ml/min was infused into the LAD before administration of glibenclamide. Baseline hemodynamic parameters were recorded for 2 min and the blood was sampled from the aortic arch and coronary vein for the measurement of SaO₂, SvO₂ and lactate concentration. Glibenclamide was then infused into the LAD at 1 ml/min while CBF at the LAD and other hemodynamic parameters were monitored continuously and recorded. Graded doses of glibenclamide at 5, 15 and 50 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ were infused. CBF and other hemodynamic parameters were allowed to stabilize for at least 2

Table 1 Effect of Glibenclamide on Coronary Blood Flow (CBF) and Other Hemodynamic Variables

Glibenclamide ($\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	CBF ($\text{ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$)	HR (beats/min)	Mean AoP (mmHg)	LVEDP (mmHg)	LV dp/dt(+) (mmHg/s)	AVOD (ml/L)
<i>Control dogs (n=10)</i>						
0	109 $\pm$ 5	161 $\pm$ 3	104 $\pm$ 6	5 $\pm$ 1	2000 $\pm$ 160	93 $\pm$ 7
5	94 $\pm$ 5 [†]	163 $\pm$ 1	104 $\pm$ 7	5 $\pm$ 2	2000 $\pm$ 150	104 $\pm$ 6
15	79 $\pm$ 5 ^{††}	163 $\pm$ 1	103 $\pm$ 7	5 $\pm$ 2	1900 $\pm$ 190	114 $\pm$ 8 [†]
50	80 $\pm$ 6 ^{††}	161 $\pm$ 2	105 $\pm$ 5	5 $\pm$ 2	1900 $\pm$ 160	121 $\pm$ 8 ^{††}
<i>Pacing dogs (n=10)</i>						
0	107 $\pm$ 5	159 $\pm$ 0	106 $\pm$ 5	7 $\pm$ 1	2200 $\pm$ 110	94 $\pm$ 14
5	90 $\pm$ 4 [†]	159 $\pm$ 0	99 $\pm$ 6	6 $\pm$ 1	2060 $\pm$ 90	108 $\pm$ 17
15	84 $\pm$ 5 ^{††}	159 $\pm$ 1	103 $\pm$ 7	5 $\pm$ 1	1880 $\pm$ 80	110 $\pm$ 19
50	78 $\pm$ 6 ^{††}	159 $\pm$ 1	102 $\pm$ 7	6 $\pm$ 1	1880 $\pm$ 120 [†]	116 $\pm$ 17 [†]
<i>Chronic heart failure dogs (n=10)</i>						
0	92 $\pm$ 4*	165 $\pm$ 0	92 $\pm$ 6	12 $\pm$ 3*	1400 $\pm$ 120*	95 $\pm$ 6
5	82 $\pm$ 6* [†]	164 $\pm$ 0	89 $\pm$ 5	12 $\pm$ 3*	1400 $\pm$ 130*	102 $\pm$ 8
15	66 $\pm$ 7* ^{††}	165 $\pm$ 0	90 $\pm$ 5	13 $\pm$ 3*	1400 $\pm$ 120*	112 $\pm$ 7 [†]
50	39 $\pm$ 6* ^{†††}	164 $\pm$ 0	86 $\pm$ 5*	15 $\pm$ 3*	1200 $\pm$ 100**	123 $\pm$ 4 ^{††}
50+NG	86 $\pm$ 4	164 $\pm$ 0	89 $\pm$ 5*	12 $\pm$ 3*	1400 $\pm$ 100**	100 $\pm$ 6

Values are mean $\pm$ SEM. NG, nitroglycerin.

* $p<0.05$, ** $p<0.01$ vs control dogs; [†] $p<0.05$, ^{††} $p<0.01$ vs before glibenclamide.

Table 2 Effect of Glibenclamide on Blood Gas and Lactate Data

Glibenclamide ($\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	Arterial			Venous			Lactate extraction ratio (%)
	pH	PaO ₂ (mmHg)	PaCO ₂ (mmHg)	pH	PvO ₂ (mmHg)	PvCO ₂ (mmHg)	
Control dogs							
0	7.36±0.02	136±15	32±2	7.32±0.02	23±1	42±2	38±5
5	7.35±0.02	126±7	29±2	7.29±0.02	18±1	41±3	Not measured
15	7.35±0.01	126±6	31±2	7.26±0.03	16±1	46±3	Not measured
50	7.36±0.02	129±8	30±2	7.25±0.03 [†]	17±1 [†]	47±3	21±8 [†]
Pacing dogs							
0	7.40±0.01	144±9	24±1	7.34±0.02	24±2	30±1	39±7
5	7.40±0.01	154±9	23±1	7.32±0.02	24±2	30±1	Not measured
15	7.38±0.02	155±9	24±1	7.27±0.03	21±1	35±1	Not measured
50	7.37±0.02	150±9	24±1	7.22±0.03 [†]	20±1 [†]	39±2 [†]	20±7 [†]
CHF dogs							
0	7.35±0.02	140±12	34±2	7.29±0.02	23±1	40±2	32±2
5	7.35±0.02	140±14	31±2	7.26±0.02	22±2	42±3	Not measured
15	7.36±0.02	140±14	29±2	7.22±0.02* [†]	20±2	49±3* ^{††}	Not measured
50	7.35±0.02	142±15	31±2	7.13±0.03* ^{†††}	18±2 [†]	58±3* ^{†††}	-99±26* ^{†††}
50+NG	7.35±0.02	142±15	31±2	7.26±0.02	20±2	40±3	23±6

Values are mean $\pm$ SEM. NG, nitroglycerin; CHF, chronic heart failure.

* $p<0.05$, ** $p<0.01$ vs control dogs; [†] $p<0.05$, ^{††} $p<0.01$ vs before glibenclamide.

min before increasing the dose of glibenclamide.

To determine whether glibenclamide-induced myocardial metabolic abnormalities resulted from reduced CBF supply, the effect of restoring CBF to the baseline level was examined in control and CHF dogs. Five min after the infusion of glibenclamide at $50\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, intracoronary infusion of nitroglycerin at $5\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ was started. After 5 min of nitroglycerin infusion, CBF and hemodynamic and metabolic parameters were measured. The SaO₂, SvO₂ and blood gas parameters were measured at the baseline, during each dose of glibenclamide, and during glibenclamide plus nitroglycerin infusion. Arterial and coronary venous lactate concentrations were measured at the baseline, during glibenclamide at $50\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and during glibenclamide plus nitroglycerin.

Efficacy of K⁺ATP channel blockade with $50\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ was evaluated by the following preliminary experiments. Glibenclamide blunted ($p<0.01$) the increases in CBF by intracoronary pinacidil at $100\mu\text{g}/\text{min}$. Glibenclamide also attenuated ($p<0.01$) the CBF response to adenosine at

$100\mu\text{g}/\text{min}$, but did not affect the CBF response to acetylcholine at $3\mu\text{g}/\text{min}$ or sodium nitroprusside at $30\mu\text{g}/\text{min}$. The pinacidil-induced increases in CBF before and after glibenclamide were $280\pm 26\%$ and $32\pm 8\%$ ($p<0.01$) in control dogs ($n=5$) and $181\pm 20\%$ and $15\pm 6\%$ ($p<0.01$) in CHF dogs ($n=5$), respectively. The adenosine-induced increases in CBF before and after glibenclamide were $390\pm 28\%$ and $133\pm 16\%$ ($p<0.01$) in control dogs and $262\pm 24\%$ and $90\pm 12\%$ ($p<0.01$) in CHF dogs. The increases in CBF by acetylcholine before and after glibenclamide were $224\pm 20\%$ and $214\pm 19\%$ in control dogs and $146\pm 18\%$ and $165\pm 16\%$ in CHF dogs. The increases in CBF by sodium nitroprusside before and after glibenclamide were $84\pm 9\%$ and $88\pm 9\%$ in control dogs and $80\pm 9\%$ and $82\pm 11\%$ in CHF dogs.

Protocol 2 Regional MBF was measured in 6 CHF dogs and 6 controls at baseline (vehicle infusion) and during glibenclamide at $50\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, using the microsphere technique. Aop, LV pressure, HR, and LVdp/dt were recorded.

Fig 1. Effects of intracoronary infusion of the K⁺ATP channel blocker glibenclamide on basal coronary blood flow (CBF) and coronary vascular resistance (CVR = mean systemic blood pressure – mean LV diastolic pressure/coronary blood flow). * $p < 0.01$ vs control dogs, † $p < 0.01$ vs Pacing dogs.

Protocol 3 Effects of NO synthase inhibition on basal CBF were examined in 5 control and 5 CHF dogs. Physiological saline vehicle at 1 ml/min was infused into the LAD before administration of a NO synthase inhibitor N^G-nitro-L-arginine (LNNA). Baseline hemodynamic parameters were recorded for 2 min. LNNA was then infused into the LAD at 2 mg/kg over a period of 10 min at a rate of 1 ml/min while CBF at the LAD and other hemodynamic parameters were monitored continuously and recorded. Efficacy of NO synthase inhibition was evaluated by the following preliminary experiments. LNNA had no effect on mean Aop, LVdp/dt or left ventricular end-diastolic pressure (LVEDP). It markedly attenuated ($p < 0.01$) the increases in CBF from intracoronary infusion of acetylcholine at 3 μg/min and did not affect the CBF response to intracoronary infusion of sodium nitroprusside at 30 μg/min. The acetylcholine-induced increases in CBF before and after LNNA were, respectively, 253±25% and 100±12% in control dogs and 163±19% and 90±12% in CHF dogs. The increases in CBF by sodium nitroprusside before and after LNNA were 85±10% and 88±10% in control dogs and

80±10% and 84±10% in CHF dogs.

Protocol 4 Effects of adenosine receptor blockade on basal CBF were examined in 5 control and 5 CHF dogs. Baseline hemodynamic parameters were recorded for 2 min. An adenosine receptor blocker 8-phenyltheophylline (8-PT) was then infused intravenously at 10 mg/kg over 10 min while CBF at the LAD and other hemodynamic parameters were monitored continuously and recorded. Efficacy of adenosine receptor blockade was evaluated by the following preliminary experiments. 8-PT had no effect on mean Aop, LVdp/dt or LVEDP, but markedly attenuated ($p < 0.01$) the increases in CBF from intracoronary adenosine at 100 μg/min. 8-PT did not affect the CBF response to intracoronary infusion of pinacidil at 100 μg/min or sodium nitroprusside at 30 μg/min. The adenosine-induced increases in CBF before and after 8-PT were, respectively, 380±30% and 30±6% ($p < 0.01$) in control dogs ($n = 5$) and 283±22% and 25±6% ($p < 0.01$) in CHF dogs ($n = 5$). The increases in CBF by pinacidil at 100 μg/min before and after 8-PT were 250±26% and 255±29% in control dogs and 144±19% and 142±15% in CHF dogs. The increases in CBF by sodium nitroprusside before and after 8-PT were 84±9% and 88±9% in control dogs and 80±9% and 82±11% in CHF dogs.

Protocol 5 Effects of intracoronary infusion of vasopressin (0.3 unit/min) was examined in 5 control and 5 CHF dogs. Baseline hemodynamic parameters were recorded for 2 min. Vasopressin was then infused into the LAD while CBF at the LAD and other hemodynamic parameters were monitored continuously and recorded.

Vasopressin caused comparable decreases in CBF in control (31±6%) and CHF dogs (29±8%), but had no effects on hemodynamic variables (data not shown).

Statistical Analysis

Data are expressed as the mean ± SEM. Paired and unpaired data were analyzed by the Students t test. When changes in hemodynamic or metabolic parameters in response to glibenclamide were compared in multiple points or between 3 groups, ANOVA followed by Bonferroni's comparison test was used. A p value of less than 0.05 was considered statistically significant.

Results

Long-term pacing tachycardia caused a significant decrease in LV ejection fraction (68±3%, 64±2%, and 28±2% in control dogs, Pacing dogs and CHF dogs, respectively) and increase in LV end-diastolic dimensions (37±1 mm, 38±1 mm and 46±3 mm in control dogs, Pacing dogs and CHF dogs, respectively) as previously reported by other investigators.^{7,18} There was no significant difference among the 3 groups in body weight, white blood cell count, serum concentrations of C-reactive protein, creatinine, blood urea nitrogen, sodium or potassium (data not shown). Blood hemoglobin concentrations were comparable between control dogs (12.3±0.6 g/dl), Pacing dogs (11.7±0.8 g/dl) and CHF dogs (12.0±0.6 g/dl).

Effects of Glibenclamide on Basal Coronary Blood Flow (Protocol 1)

The baseline CBF, Aop, and LV dp/dt were significantly lower and the LVEDP were higher in HF dogs than in control dogs (Tables 1, 2). There were no significant differences in those variables between control and Pacing dogs.

Glibenclamide decreased CBF in all 3 groups (Fig 1).

Table 3 Effects of Glibenclamide on Myocardial Blood Flow

	Control dogs (n=6) glibenclamide		CHF dogs (n=6) glibenclamide	
	Before	After	Before	After
<i>MBF (ml·min⁻¹·g⁻¹)</i>				
<i>LAD area to which glibenclamide was infused</i>				
ENDO	1.52±0.16	0.97±0.13**	1.14±0.11 [†]	0.54±0.13***
MID	1.60±0.24	1.14±0.21**	1.28±0.17 [†]	0.57±0.12***
EPI	1.19±0.17	0.84±0.08**	1.40±0.12 [†]	0.58±0.12***
Mean	1.48±0.15	0.97±0.13**	1.21±0.11 [†]	0.57±0.11***
I/O ratio	1.32±0.13	1.14±0.09	0.83±0.05 [†]	1.07±0.19
<i>LCX area without glibenclamide infusion</i>				
ENDO	1.48±0.14	1.47±0.25	0.96±0.14 [†]	1.21±0.23 [†]
MID	1.40±0.24	1.27±0.26	1.16±0.46	1.31±0.78
EPI	1.20±0.14	1.03±0.18	1.21±0.10 [†]	1.07±0.16
Mean	1.40±0.18	1.25±0.19	1.08±0.16 [†]	1.20±0.26
I/O ratio	1.23±0.11	1.37±0.24	0.83±0.10 [†]	1.32±0.23
HR (beats/min)	160±1	160±1	160±1	160±1
Mean AoP (mmHg)	102±9	106±7	92±6 [†]	90±6 [†]
LV dp/dt (mmHg/s)	2000±160	2100±140	1410±110 [†]	1400±110 [†]
LVEDP (mmHg)	3±1	3±1	15±2 [†]	17±2 [†]

Data are mean±SEM. MBF, myocardial blood flow; HR, heart rate.

**p<0.01 vs before glibenclamide infusion; [†]p<0.05 vs control dogs.

The magnitudes of decreases in CBF in responses to glibenclamide at 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ were significantly greater in CHF dogs than in control or Pacing dogs ($p<0.01$) whereas the CBF responses to glibenclamide at 5 and 15 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ did not significantly differ among the 3 groups. Glibenclamide at 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ increased ($p<0.01$) AVOD in the 3 groups (Table 1).

During vehicle infusion, there were no significant differences in arterial and coronary venous pH, PO_2 , PCO_2 and the lactate extraction ratio ($100\times[\text{lactate concentration in arterial blood}-\text{lactate concentration in coronary venous blood}]/\text{lactate concentration in arterial blood}$) among the 3 groups. Glibenclamide at 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ significantly decreased the lactate extraction ratio to below zero in CHF dogs, but not in control and Pacing dogs (Table 2). Myocardial lactate production was induced in all of 10 CHF dogs by glibenclamide at 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. This was associated with a decrease in the pH and an increase in PCO_2 in coronary venous blood.

Restoring CBF to the baseline level by nitroglycerin normalized myocardial lactate production and acidosis (Tables 1, 2).

Effects of Glibenclamide on Regional MBF (Protocol 2)

Before glibenclamide, the basal subendocardial MBF was significantly less and the subepicardial MBF was greater in CHF dogs than controls with no significant difference of mean MBF between the 2 groups (Table 3). The I/O ratio (subendocardial MBF/subepicardial MBF) was thus significantly less in CHF dogs than in controls as previously reported by others^{17,18}. Glibenclamide at 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ significantly decreased MBF in subendocardial, midmyocardial and subepicardial layers in both CHF dogs and controls. The glibenclamide-induced decreases in MBF at any myocardial layers were significantly greater in CHF dogs than in controls as observed in Protocol 1. Thus, glibenclamide did not affect the I/O ratio in control and CHF dogs.

Effects of LNNA and 8-PT on Basal Coronary Blood Flow (Protocols 3 and 4)

LNNA did not cause a significant change in CBF in

control (102±9 to 98±10 $\text{ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$) and CHF dogs (93±10 to 95±11 $\text{ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$). Also, 8-PT did not cause a significant decrease in CBF in control (104±11 to 108±10 $\text{ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$) and HF dogs (96±6 to 95±8 $\text{ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$). Neither LNNA nor 8-PT caused significant changes in mean AoP, LVP, LVdp/dt or LVEDP in control and CHF dogs (data not shown).

Discussion

The major new finding of the present study is that the decreases in CBF with glibenclamide were greater in CHF dogs than controls. In addition, the greater decrease in CBF was associated with myocardial lactate production suggestive of the development of myocardial ischemia. These findings suggest that coronary vascular K⁺ATP channel activity is increased in CHF dogs than in controls.

Effects of Glibenclamide on Basal CBF in CHF

It has been shown in intact anesthetized or conscious dogs that intracoronary infusion of glibenclamide at the doses ranged from 5 to 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ decreased CBF by 10–40% in a dose-dependent manner without altering myocardial oxygen consumption (refs 12, 24, 25 and the present study). We found in the present study that the magnitude of the decrease in CBF at 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ was greater in CHF dogs than controls. It is unlikely that changes in hemodynamic parameters accounted for the enhanced effects of glibenclamide, because AoP, LVdp/dt, LVEDP and HR during glibenclamide infusion did not differ from those during vehicle infusion. The glibenclamide-induced decrease in CBF in CHF was associated with the increase in myocardial oxygen extraction (AVOD), suggesting that the enhanced effect of glibenclamide did not result from the decrease in myocardial oxygen consumption or from lower systemic blood pressure in CHF dogs. Thus, the enhanced decrease in CBF with glibenclamide resulted in inadequate oxygen supply to the myocardium and thus caused myocardial metabolic abnormalities such as myocardial lactate production. Furthermore, we observed that glibenclamide-induced myocardial metabolic abnor-

malities were reversed when CBF levels were restored to the baseline levels during vehicle infusion with nitroglycerin. These findings support the hypothesis that glibenclamide caused a primary decrease in CBF with a secondary impairment of myocardial metabolic abnormalities.

In addition to K⁺ATP channels, other endogenous vasodilator systems, such as NO or adenosine, contribute to the regulation of CBF in physiological and/or pathophysiological states.^{13,24,25} We thus examined the effects of NO synthase inhibition and adenosine receptor blockade and found that neither caused a decrease in CBF in control or CHF dogs. These findings suggest that relative to NO or adenosine, K⁺ATP channel activity in the coronary circulation is of functional importance in maintaining basal CBF in CHF.

Because short-term rapid pacing can activate cardiovascular K⁺ATP channels,²⁶ we studied another group of dogs (Pacing dogs), which underwent rapid pacing for only 1 week but did not manifest CHF. The basal hemodynamic or metabolic status or the CBF response to glibenclamide in this group were comparable to control dogs, suggesting that rapid pacing per se does not account for altered effects of glibenclamide in CHF dogs.

MBF in the subendomyocardial layers is jeopardized in animals and humans with CHF,^{17,18,27} which may result at least in part from the increase in extravascular compressive forces.^{28,29} Shannon et al reported that impaired subendocardial MBF at rest and during adenosine infusion was restored when LVEDP was normalized in dogs with pacing-induced CHF.¹⁸ Several studies have shown that hypoperfusion or ischemia activates vascular K⁺ATP channels.⁷⁻⁹ In the present study, we observed subendocardial hypoperfusion (reduced I/O ratio) at rest in CHF dogs and that the glibenclamide-induced decrease in MBF did not differ among the 3 myocardial layers, which suggests that glibenclamide decreased MBF to a similar extent in the subendocardial and subepicardial layers.

Possible Mechanisms for K⁺ATP Channel Activation in CHF

The mechanisms for K⁺ATP channel activation were not explored in the present study, but they must be multifactorial and include gene/receptor modulation, nucleotide concentrations and physical environment. Akao et al recently demonstrated in a rat model of regional myocardial ischemia that prolonged myocardial ischemia induced upregulation of a putative K⁺ATP channel (Kir6.1) in ischemic and nonischemic myocardium.³⁰ They suggested that myocardial mechanical overload as well as myocardial ischemia cause this upregulation. Decreased adenine nucleotide concentrations and/or reduced channel sensitivity may also contribute to increased K⁺ATP channel activity in CHF. Intracellular adenine nucleotide concentrations (ATP or ATP/ADP ratio) in the heart are reduced in the presence of CHF.³¹ Furthermore, K⁺ATP channels are reported to be less sensitive to ATP inhibition in cardiac myocytes from patients with CHF³² or from rats with myocardial hypertrophy/failure.²⁰ In addition, K⁺ATP channel activity may be regulated also by adenosine binding to the channel's extracellular regulatory sites.^{2,3} The coronary vasodilation induced by the K⁺ATP channel opener pinacidil or by hypoxia is blocked by glibenclamide, but not by 8-PT (ref 24, 25 and present study). In contrast, the coronary vasodilation induced by adenosine is attenuated by glibenclamide and 8-PT.^{24,25,33,34} These observations suggest that adenosine

causes coronary vasodilation via activation of coronary vascular K⁺ATP channels. Plasma adenosine concentrations are reported to increase in CHF³⁵ thus it is likely that in CHF, perivascular accumulation of adenosine may increase the activity of K⁺ATP channels. Finally, a complex of factors including ischemia/hypoxia,⁹ acidosis,³⁶ increased lactate production,³⁷ mechanical stress to the cell membrane² and so forth, all of which occurs during CHF, may activate the cardiac and vascular K⁺ATP channels.

No matter what is the mechanism of coronary vascular K⁺ATP channel activation, our present observations suggest that a higher level of basal coronary tone (ie, myocardial hypoperfusion) is an intrinsic characteristic of the coronary vascular bed in CHF. It is likely that increased activity of K⁺ATP channels is one of the important compensatory mechanisms for this increased vascular tone.

Study Limitations

We used anesthetized open-chest preparations where general anesthesia and/or acute surgery could cause coronary vasodilation associated with a higher level of coronary venous oxygen tension. Under the condition of higher oxygen tension in the coronary vein, glibenclamide can produce a greater decrease in CBF, even in intact dogs, as seen in some previous studies.^{5,6} To minimize these adverse effects, we made the inclusion criteria described in the Methods section. Accordingly, the myocardial metabolic states (arterial and coronary venous pH, oxygen pressure and bicarbonate pressure) were within the physiological range and similar between CHF dogs and controls (Table 2). A 20–30% decrease in basal CBF in the control dogs with glibenclamide at 50 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the present study is comparable to the data in intact conscious dogs reported by Duncker et al.¹² Furthermore, there was no correlation between the degree of decrease in basal CBF by glibenclamide and basal oxygen tension in the coronary vein. However, we can not exclude the possibility that our present observations were at least in part the results of the presence of anesthesia.³⁸

Another caveat is that the greater decrease in CBF was noted only at the high dose of glibenclamide (50 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Thus, we cannot exclude the remote possibility that glibenclamide at the high dose produced non-specific actions.

The interpretation of the results of previous studies and the present study, which investigated the role of K⁺ATP channels in the regulation of CBF in vivo, rely on the assumption that glibenclamide is a specific inhibitor of K⁺ATP channels in the coronary vasculature. Glibenclamide has been shown to produce a high degree K⁺ATP channel blockade without affecting the coronary vasodilation induced by sodium nitroprusside or acetylcholine.^{10,11,14,24} The closure of K⁺ATP channels with glibenclamide causes depolarization of vascular smooth muscle membrane and vasoconstriction.^{2,3} Thus, glibenclamide may cause vasoconstriction by blocking signals of the endogenous vasodilators that cause vasodilation by opening K⁺ATP channels.

Summary

The basal opening state or activity of coronary vascular K⁺ATP channels is increased in CHF dogs than in controls, which may contribute to the maintenance of basal myocardial perfusion in HF.

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