Effect of $K^{+}_{ATP}$ Channel and Adenosine Receptor Blockade During Rest and Exercise in Congestive Heart Failure

Jay H. Traverse, YingJie Chen, MingXiao Hou, Yunfang Li, Robert J. Bache

Abstract—$K^{+}_{ATP}$ channels are important metabolic regulators of coronary blood flow (CBF) that are activated in the setting of reduced levels of ATP or perfusion pressure. In the normal heart, blockade of $K^{+}_{ATP}$ channels results in a $\approx 20\%$ reduction in resting CBF but does not impair the increase in CBF that occurs during exercise. In contrast, adenosine receptor blockade fails to alter CBF or myocardial oxygen consumption (MVO$_2$) in the normal heart but contributes to the increase in CBF during exercise when vascular $K^{+}_{ATP}$ channels are blocked. Congestive heart failure (CHF) is associated with a decrease in CBF that is matched to a decrease in MVO$_2$ suggesting downregulation of myocardial energy utilization. Because myocardial ATP levels and coronary perfusion pressure are reduced in CHF, this study was undertaken to examine the role of $K^{+}_{ATP}$ channels and adenosine in dogs with pacing-induced CHF. Myocardial blood flow (MBF) and MVO$_2$ were measured during rest and treadmill exercise before and after $K^{+}_{ATP}$ channel blockade with glibenclamide ($50\ \mu g/kg/min$ ic) or adenosine receptor blockade with 8-phenyltheophylline (8-PT; 5 mg/kg iv). Inhibition of $K^{+}_{ATP}$ channels resulted in a decrease in CBF and MVO$_2$ at rest and during exercise without a change in the relationship between CBF and MVO$_2$. In contrast, adenosine receptor blockade caused a significant increase in CBF that occurred secondary to an increase of MVO$_2$. These findings demonstrate that coronary $K^{+}_{ATP}$ Channel activity contribute to the regulation of resting MBF in CHF, and that endogenous adenosine may act to inhibit MVO$_2$ in the failing heart. (Circ Res. 2007;100:1643-1649.)

Key Words: heart failure $\bullet$ $K^{+}_{ATP}$ channels $\bullet$ coronary blood flow $\bullet$ exercise $\bullet$ myocardial oxygen consumption

$K^{+}_{ATP}$ channels exist on the sarcolemma of vascular smooth muscle cells and endothelium where they can contribute to regulation of coronary blood flow (CBF). Thus, blockade of $K^{+}_{ATP}$ channels in awake dogs resulted in $\approx 20\%$ decrease of resting CBF with development of contractile dysfunction attributable to oxygen supply-demand mismatch. However, $K^{+}_{ATP}$ channel blockade did not impair the increase in CBF that occurred during exercise, indicating that $K^{+}_{ATP}$ channels are not required for the increase in metabolic coronary vasodilation.

Adenosine is a potent coronary vasodilator produced in cardiac myocytes by the catabolism of adenine nucleotides. Locally produced adenosine can interact with specific receptors on coronary endothelial and smooth muscle cells, and contribute to coronary vasodilation during myocardial ischemia. However, in the normal heart blockade of adenosine receptors fails to impair vasodilator responses to increases of myocardial oxygen demands caused by exercise, or tachycardia, indicating that adenosine is not required for metabolic coronary vasoregulation.

We have previously observed that CBF and MVO$_2$ are reduced in dogs with pacing-induced congestive heart failure (CHF) compared with normal controls. Coronary sinus $pO_2$ was unchanged and oxygen extraction was not increased in the failing hearts, implying that the reduction in MVO$_2$ did not result from insufficient oxygen availability, but rather that oxygen demands are reduced in the failing heart. In open-chest dogs with CHF, blockade of vascular $K^{+}_{ATP}$ channels with glibenclamide produced a significant decrease in CBF demonstrating a contribution of $K^{+}_{ATP}$ channel activity to the maintenance of resting blood flow in the failing heart. Therefore, the first part of this study was undertaken to determine the role of coronary $K^{+}_{ATP}$ channel activity in the failing heart in conscious dogs during rest and exercise.

Several investigators have reported that cardiac adenosine levels are increased in CHF. Thus, pericardial levels of adenosine were 3- to 4-fold higher than control in dogs with CHF secondary to an aortocaval fistula, and plasma adenosine levels were increased in patients with CHF. The mechanism for the increase in adenosine levels is not known, although it has been postulated that increased norepinephrine and angiotensin II in CHF may activate ecto-5'-nucleosidase, which is responsible for adenosine formation through a protein kinase C-dependent mechanism. Based on these previous findings, a second aim of this study was to determine whether the increased adenosine levels exert...
a physiological effect on the coronary circulation in the failing heart.

Materials and Methods

Surgical Instrumentation and Production of CHF
Surgical instrumentation was performed in 28 adult mongrel dogs (25 to 30 kg) in accordance with the "Position of the American Heart Association on Research Animal Use" and approved by the Animal Care Committee of the University of Minnesota as previously described. CHF was produced by rapid ventricular pacing. One week after surgery the pacemaker was activated at 220 bpm. This rate was continued or increased to 250 bpm if evidence of CHF was not present within 3 weeks. Resting hemodynamics and CBF were assessed weekly in normal sinus rhythm 1 hour after the pacemaker was deactivated. CHF was deemed to have developed when LV end-diastolic pressure was >20 mm Hg. Five dogs died suddenly during the pacing protocol, presumably secondary to a lethal arrhythmia, and 3 dogs could not be studied because of loss of the flow probe signal. A total of 20 dogs completed the pacing protocol and subsequently underwent measurements of CBF and MVO2 in response to vascular K*ATP channel blockade (n=11) or adenosine receptor blockade (n=9) during rest and exercise.

Effect of K*ATP Channel Blockade With Glibenclamide on CBF and MVO2
Eleven dogs with CHF were studied to examine the role of vascular K*ATP channels in regulating CBF and MVO2 during rest and exercise. One hour after the pacemaker was deactivated, resting hemodynamics, blood flow, and blood gases were obtained with the dogs standing quietly on the treadmill. The treadmill was activated and the dogs began to exercise at 3.2 km/h and 0% grade for 4 minutes. Hemodynamics and blood flow were continuously recorded. During the last 30 seconds of exercise aortic and coronary sinus blood was withdrawn for blood gas analysis. Coronary K*ATP channel blockade was performed by infusing glibenclamide at a dose of 50 μg/kg/min and rate of 1.5 mL/min through the intracoronary catheter 2 hours after completion of the Control run. After a 5 minute infusion, resting hemodynamics, blood flow, and blood gases were obtained. The treadmill was then activated and the exercise protocol was repeated in an identical manner to the Control study.

Magnitude and Selectivity of K*ATP Channel Blockade Produced by Glibenclamide
To determine the selectivity of glibenclamide to inhibit coronary vascular K*ATP channel activity, coronary flow responses to increasing doses of the K*ATP channel opener pinacidil was measured in 4 dogs before and after K*ATP channel blockade. With the dogs standing quietly in a sling, pinacidil was infused through the intracoronary catheter at doses of 0.5 to 10 μg/kg/min at infusion rates of 0.15 to 3.0 mL/min. Glibenclamide (50 μg/kg/min) was then infused through the catheter over 5 minutes and the measurements to pinacidil were repeated.

Effects of Adenosine Receptor Blockade With 8-Phenyltheophylline (8-PT) on CBF and MVO2
A total of 9 dogs with CHF were studied to examine the role of adenosine receptor blockade on CBF and MVO2 during rest and exercise. On the day of study, hemodynamics and CBF were recorded continuously beginning 1 hour after pacemaker deactivation with the dog standing on the treadmill. Two mL of aortic and coronary venous blood were withdrawn on ice for determination of resting MVO2. The dogs began to exercise at 3.2 km/hr at 0% grade for a total of 4 minutes. Hemodynamics and blood flow were continuously recorded, and aortic and coronary venous blood specimens were withdrawn for blood gas analysis during the last 30 seconds of exercise. After completion of baseline exercise the dogs were allowed to rest for 2 hours. The adenosine receptor blocker 8-phenyltheophylline (8-PT) was then administered in a dose of 5 mg/kg through the left atrial catheter over 15 minutes. Thirty minutes later the study was repeated.

Effect of 8-PT on the CBF Response to Adenosine
On a separate day the effectiveness of 8-PT in blocking the coronary response to adenosine was examined in 3 dogs with CHF. Adenosine was administered through the LAD catheter at doses of 0 to 100 μg/kg/min (infusion rates of 0.15 to 3.0 mL/min) before and after 8-PT (5 mg/kg iv) as described above.

Measurement of Myocardial Blood Flow
Mean myocardial blood flow (MBF) was measured during control exercise and exercise after K*ATP channel and adenosine receptor blockade using 15 μm diameter radioactive microspheres (NEN Co) as previously described. For each measurement 3×10⁶ microspheres were injected into the left atrium and flushed with saline. A reference sample of arterial blood was obtained from the aortic catheter at a constant rate of 15 mL/min beginning at the time of injection and continuing for 90 seconds. After completion of the studies the animals were euthanized with an overdose of pentobarbital and the heart was removed and fixed in formalin. Based on previous staining of the LAD region, the anterior and septal regions were taken as the LAD perfusion territory. These regions were weighed and placed in vials for counting in a gamma spectrometer (Packard Instrument Co) at window settings corresponding to the peak energies of each radionuclide. Resting MBF measurements were calculated by calibration of the resting LAD flow probe signal to that measured during exercise normalized to the exercise MBF in the region of myocardium perfused by the LAD.

Myocardial Oxygen Consumption
Aortic and coronary venous pO₂, pCO₂, and pH were determined with a blood gas analyzer (Instrumentation Laboratory Model 113). Blood oxygen content was calculated as (0.0136×hemoglobin×% oxygen saturation)+(pO₂×0.0031). Oxygen consumption on a per gram basis in the LAD myocardial region was calculated as the MBF multiplied by the arteriovenous difference in oxygen content.

Data Analysis
Heart rate, pressures, and coronary velocity were measured from the strip chart recordings. CBF was calculated from the Doppler frequency shift (kHz) as previously described. LV dp/dt was obtained by electrical differentiation of the LV pressure signal. Coronary vascular resistance (CVR) was calculated as: (Ao−LVEDP)/CBF. Data were compared within and between Control and CHF groups by ANOVA for repeated measures; a value of P<0.05 was considered significant. When a significant result was found, individual comparisons were performed with the Wilcoxon signed-rank test.

Drugs
Glibenclamide and 8-PT were purchased from Sigma. Glibenclamide and 8-PT were dissolved in 0.5N NaOH and dH₂O and heated into solution.

Results

Effect of K*ATP Channel Blockade on Coronary Blood Flow and Hemodynamics
In the 11 dogs that underwent K*ATP channel blockade, the production of CHF resulted in significant reductions in mean arterial pressure (84±2 mm Hg) and increases in resting HR (135±3 bpm) and LVEDP (24±2 mm Hg) compared with normal dogs previously studied in our laboratory (Table 1). Resting MBF in the LAD region was 1.03±0.12 mL/min-g. During Control exercise there was a significant increase in mean arterial pressure (96±3 mm Hg), heart rate (184±3
was further increased as the CS pO2 decreased to 16.5 mm Hg.

Channel blockade caused a parallel and leftward shift in this vasodilation (Figure 1A).

Glibenclamide administration decreased resting MBF to 0.57 ± 0.09 mL/min (P < 0.05), suggesting a deterioration in left-ventricular function as previously demonstrated in normal dogs after K^\text{ATP} channel blockade.1 Glibenclamide administration decreased resting MBF to 0.57 ± 0.09 mL/min (P < 0.01) and resulted in a significant increase in CVR (1.8 ± 0.1 to 3.0 ± 1.2 mm Hg/mL/min). During exercise, MBF after K^\text{ATP} channel blockade was significantly reduced compared with Control (P < 0.05), although the increase in CBF between rest and exercise was not different (Table 1).

### Effect of K^\text{ATP} Channel Blockade on Myocardial Oxygen Consumption

MVO_2 was measured in 5 dogs in which coronary sinus catheters remained patent during the development of CHF. Resting MVO_2 was 95 ± 12 μL O_2/min-g which decreased to 67 ± 17 μL O_2/min-g after vascular K^\text{ATP} channel blockade (Table 2). This was primarily a result of the decrease in MBF with only a small increase in oxygen extraction. During Control exercise, MVO_2 increased significantly to 149 ± 12 μL O_2/min-g (P < 0.05) and was accompanied by an increase in oxygen extraction (CS pO_2 decreased from 25 ± 5 to 19.5 ± 3 mm Hg; P < 0.05). After glibenclamide, MVO_2 increased during exercise to 121 ± 17 μL O_2/min-g which was significantly less than Control exercise. Oxygen extraction was further increased as the CS pO_2 decreased to 16.5 ± 4 mm Hg (P < 0.05 versus Control). Plotting coronary venous oxygen tension versus MVO_2 demonstrated that K^\text{ATP} channel blockade caused a parallel and leftward shift in this relationship consistent with its inhibition of resting metabolic vasodilation (Figure 1A).

**TABLE 1. Hemodynamic Data From 11 Dogs With CHF at Rest and During Treadmill Exercise During Control Conditions (CON) and After Vascular K^\text{ATP} Channel Blockade With Glibenclamide (GLIB; 50 μg/kg/min i.c.)**

<table>
<thead>
<tr>
<th>Heart Rate (beats/min)</th>
<th>Mean Arterial Pressure (mm Hg)</th>
<th>LV Systolic Pressure (mm Hg)</th>
<th>LV End Diastolic Pressure (mm Hg)</th>
<th>MBF (mL/min-g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON 135±3</td>
<td>84±2</td>
<td>109±4</td>
<td>24±2</td>
<td>1.03±0.12</td>
</tr>
<tr>
<td>GLIB 138±7</td>
<td>86±4</td>
<td>110±5</td>
<td>31±1*</td>
<td>0.57±0.09*</td>
</tr>
<tr>
<td>EX 184±3</td>
<td>96±3</td>
<td>126±5</td>
<td>32±2</td>
<td>1.24±0.12</td>
</tr>
</tbody>
</table>

*P<0.05 vs CON.

**TABLE 2. Hemoglobin, Coronary Venous Oxygen Tension, and Myocardial Oxygen Consumption in 5 Dogs With CHF at Rest and During Treadmill Exercise**

<table>
<thead>
<tr>
<th>Hemoglobin (mg/dl)</th>
<th>Coronary Venous PO_2 (mm Hg)</th>
<th>MVO_2 (μL O_2/min-g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON 9.4±1.0</td>
<td>25±5</td>
<td>95±12</td>
</tr>
<tr>
<td>GLIB 9.7±0.8</td>
<td>23±3</td>
<td>67±17*</td>
</tr>
<tr>
<td>EX 10.7±0.9</td>
<td>19.5±3</td>
<td>149±12</td>
</tr>
</tbody>
</table>

*Data were obtained during control conditions (CON) and after K^\text{ATP} channel blockade with glibenclamide (GLIB; 50 μg/kg/min i.c.).

*P<0.05 vs CON.

During resting conditions, glibenclamide produced no significant changes in resting mean arterial pressure or heart rate but significantly increased LVEDP to 31 ± 1 mm Hg (P < 0.05), suggesting a deterioration in left-ventricular function as previously demonstrated in normal dogs after K^\text{ATP} channel blockade.1 Glibenclamide administration decreased resting MBF to 0.57 ± 0.09 mL/min (P < 0.01) and resulted in a significant increase in CVR (1.8 ± 0.1 to 3.0 ± 1.2 mm Hg/mL/min). During exercise, MBF after K^\text{ATP} channel blockade was significantly reduced compared with Control (P < 0.05), although the increase in CBF between rest and exercise was not different (Table 1).

### Effect of K^\text{ATP} Channel Blockade With Glibenclamide

Intracoronary infusions of the K^\text{ATP} channel opener pinacidil resulted in dose-dependent increases in CBF from 31 ± 1 mL/min at baseline to 84 ± 15 mL/min at the highest dose with no significant change in aortic pressure or heart rate. After infusion of glibenclamide, baseline CBF decreased to 20 ± 2 mL/min and increased only to 31 ± 2 mL/min at the highest dose of pinacidil (Figure 2A).

### Effect of Adenosine Receptor Blockade on Coronary Blood Flow and MVO_2

In 9 dogs with CHF, adenosine receptor blockade with 8-PT produced a small increase in resting heart rate but otherwise had no effect on hemodynamic variables during rest or exercise. As shown in Table 3, 8-PT resulted in a 24% increase in resting CBF that was maintained during exercise (P < 0.05). MBF increased from 1.51 ± 0.12 to 1.80 ± 0.15 mL/min/g during exercise after 8-PT.

Resting MVO_2 in the LAD region was 90 ± 12 μL O_2/min-g, whereas coronary venous PO_2 was 26 ± 4 mm Hg (n = 6). MVO_2 increased during exercise to 106 ± 15 μL O_2/min-g, whereas coronary venous PO_2 decreased to 19 ± 3 mm Hg (Table 4). 8-PT caused a significant increase in oxygen extraction with decreases of coronary venous PO_2 during rest and exercise (P < 0.05). As a result of the increases in both coronary flow and oxygen extraction, 8-PT caused significant increases of MVO_2 both at rest and during exercise (Table 4), with a 26 ± 4% increase in mean MVO_2 during rest. The slope of the coronary venous oxygen tension versus MVO_2 (Figure 1B) was unchanged after adenosine receptor blockade implying that the decrease in coronary venous O_2 tension was appropriate for the observed increase in O_2 consumption.

### Effectiveness of Adenosine Blockade With 8-PT

Intracoronary adenosine caused dose-dependent increases in CBF. 8-PT markedly blunted this response with 72% inhibi-
tion of the coronary flow response to the highest dose of adenosine (Figure 2B).

**Discussion**

This represents the first report examining vascular K<sup>+</sup><sub>ATP</sub> channel and adenosine receptor influences on myocardial blood flow and MVO<sub>2</sub> at rest and during exercise in the failing heart. Our findings demonstrate that coronary K<sup>+</sup><sub>ATP</sub> channel activity contributes significantly to the maintenance of resting MBF in the failing heart, in agreement with previous observations in the normal heart. Conversely, adenosine receptor blockade with 8-PT caused an unexpected increase in MBF that appeared to be secondary to an increase in MVO<sub>2</sub>. This suggests that, in contrast to the normal heart, adenosine exerts an inhibitory effect on O<sub>2</sub> uptake in the failing heart.

**Role of Vascular K<sup>+</sup><sub>ATP</sub> Channel Activity in the Normal Heart**

K<sup>+</sup><sub>ATP</sub> channels on the cell membrane of vascular smooth muscle are endowed with metabolic sensor activity that allows them to respond to local levels of ATP/ADP or redox potential. We and others previously reported that in the normal heart blockade of K<sup>+</sup><sub>ATP</sub> channels with intracoronary glibenclamide resulted in a 12 to 20% decrease in resting MBF. Myocardial O<sub>2</sub> extraction increased, but this was insufficient to compensate for the decrease in coronary flow, so that the decreased coronary flow caused by glibenclamide resulted in contractile dysfunction. The decrease in MVO<sub>2</sub> produced by glibenclamide is likely secondary to its marked vasoconstrictive effects as opposed to a direct effect on MVO<sub>2</sub>, because restoration of CBF with sodium nitroprusside normalizes contractile function. Despite the decrease in resting CBF, glibenclamide did not inhibit coronary vasodilation in response to exercise, implying that other vasodilator mechanisms compensate for the loss of vascular K<sup>+</sup><sub>ATP</sub> channel activity. This was supported by the finding that the addition of adenosine receptor blockade in the presence of glibenclamide inhibited coronary vasodilation during exercise. Although adenosine receptor blockade had no effect on basal coronary flow or the coronary vasodilation during exercise in the normal heart, adenosine does contribute to coronary vasodilation in the setting of ischemia when blood flow is limited by a coronary stenosis, in agreement with studies demonstrating that ischemia causes increased myocardial adenosine release. Adenosine exerts part of its vasodilator effect through activation of vascular K<sup>+</sup><sub>ATP</sub> channels, but can also evoke vasodilation through other mechanisms. Thus, low concentrations of adenosine activate K<sup>+</sup><sub>ATP</sub> channels on endothelial cells via a PTX-sensitive G protein to

**Figure 1.** A, Plot of mean coronary venous pO<sub>2</sub> vs MVO<sub>2</sub> during rest and exercise in 5 dogs before and after K<sup>+</sup><sub>ATP</sub> channel blockade with Glibenclamide, demonstrating no change in the slope of the relationship indicating that K<sup>+</sup><sub>ATP</sub> channel activation is not required for metabolic vasodilation during exercise. The decrease in coronary venous oxygen tension by glibenclamide at each level of MVO<sub>2</sub> is consistent with K<sup>+</sup><sub>ATP</sub> channel tonic vasodilatory effect in the coronary circulation during rest and exercise. B, Plot of mean coronary venous pO<sub>2</sub> vs MVO<sub>2</sub> during rest and exercise in 6 dogs before and after adenosine receptor blockade with 8-PT, demonstrating a rightward and parallel shift in the relationship following adenosine receptor blockade. Error bars represent ±SEM.

**Figure 2.** A, Coronary flow responses to the K<sup>+</sup><sub>ATP</sub> channel opener pinacidil in 5 dogs with CHF before and after infusion of the K<sup>+</sup><sub>ATP</sub> channel blocker Glibenclamide (50 µg/kg/min ic). B, Coronary flow responses to intracoronary adenosine in 3 dogs with CHF during control conditions and after adenosine receptor blockade with 8-phenyltheophylline (8-PT; 5 mg/kg iv). Error bars represent ±SEM.
cause NO release with cGMP-mediated vasodilation. Adenosine can also activate adenylyl cyclase to increase levels of cAMP in vascular smooth muscle. Furthermore, local accumulation of adenosine may be sufficient to partially overcome the $K_{ATP}$ channel blockade produced by glibenclamide.

### Role of Vascular $K_{ATP}$ Channels in the Failing Heart

CHF is associated with neurohormonal activation, elaboration of circulating vasoconstrictors, and increases in extravascular compressive forces that have the potential to limit MBF. $K_{ATP}$ channels are sensitive to decreased ATP levels near the sarcolemma of vascular smooth muscle cells and to reductions in perfusion pressure. Myocardial levels of ATP are reduced in CHF, although it is not known whether this is associated with reduced perivascular levels. However, coronary perfusion pressures are reduced in CHF as a result of a decrease in mean aortic pressure and elevations in LVEDP. Consequently, this study was performed to determine the significance of vascular $K_{ATP}$ channel activity in the failing heart. We found that $K_{ATP}$ channel blockade with glibenclamide significantly decreased resting MBF in dogs with CHF and resulted in a marked increase in CBF. This decrease in CBF was accompanied by a 30% reduction in coronary venous $pO_2$. As in the normal heart, $K_{ATP}$ channel blockade in open-chest, anesthetized dogs with pacing-induced CHF. Using the dose of glibenclamide used in the present study, they also observed a significant reduction in basal coronary blood flow in dogs with CHF. This decrease in CBF was accompanied by lactate production in the dogs with CHF, consistent with ischemia. In unanesthetized swine with moderate LV dysfunction, glibenclamide (3 mg/kg iv) produced a similar decrease in resting CBF compared with a normal control group. However, during exercise the dependence on vascular $K_{ATP}$ channel activity was enhanced in the postinfarction group compared with the control group. These findings suggest that $K_{ATP}$ channels may have increased importance for maintaining coronary vasodilation in failing or remodelled hearts. A principal cause for this finding may arise from increased extravascular compressive forces resulting in reduced perfusion pressure in the failing ventricle. Elevations in LVEDP may reduce coronary inflow as they are strongly correlated with extravascular compressive forces that create an effective back-pressure to coronary perfusion. In a previous study using this model of CHF we observed that the zero-flow pressure ($P_{ZF}$) was significantly higher than normal controls (27 versus 12 mm Hg) and highly correlated with LVEDP. $K_{ATP}$ channels in explanted failing human hearts are less sensitive to inhibition by ATP than normal. In isolated human myocytes protein kinase C (PKC) activates $I_{KATP}$ by reducing channel sensitivity to ATP. If vascular channel sensitivity to ATP is also reduced by increased PKC activity in the failing heart, than this may also help to explain the significant dependence of resting coronary flow on $K_{ATP}$ channels in the failing hearts in the present study.

### Role of Adenosine in the Normal Heart

At least 4 subtypes of adenosine receptors have been identified in the heart ($A_1$, $A_2A$, $A_2B$, and $A_3$). $A_1$ receptor is found exclusively on cardiomyocytes and mediates the inhibitory actions of adenosine on contractile performance that is opposed by $A_{2A}$ receptor activation that enhances inotropy.

### TABLE 3. Hemodynamic Data From 9 Dogs With CHF at Rest and During Treadmill Exercise During Control Conditions (CON) and After Adenosine-Receptor Blockade With 8-Phenyloethopine (8-PT; 5 mg/kg i.v.)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Heart Rate (beats/min)</th>
<th>Mean Arterial Pressure (mm Hg)</th>
<th>LV Systolic Pressure (mm Hg)</th>
<th>LV End Diastolic Pressure (mm Hg)</th>
<th>LV dP/dt (mm Hg/sec)</th>
<th>MBF (ml/min-g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>139±6</td>
<td>84±4</td>
<td>104±4</td>
<td>23±2</td>
<td>1450±340</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>EX</td>
<td>168±5</td>
<td>93±5</td>
<td>113±6</td>
<td>29±2</td>
<td>1960±575</td>
<td>1.6±0.2</td>
</tr>
</tbody>
</table>

Data were obtained during control conditions (CON) and after adenosine receptor blockade with 8-Phenyloethopine (8-PT; 5 mg/kg i.v.).

### TABLE 4. Hemoglobin, Coronary Venous Oxygen Tension, and Myocardial Oxygen Consumption in 6 Dogs With CHF at Rest and During Treadmill Exercise

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hemoglobin (mg/dl)</th>
<th>Coronary Venous $pO_2$ (mm Hg)</th>
<th>MVO$_2$ ($\mu$L O$_2$/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>9.3±0.7</td>
<td>26±4</td>
<td>23±5</td>
</tr>
<tr>
<td>EX</td>
<td>10.8±0.7</td>
<td>19±3</td>
<td>16±3</td>
</tr>
</tbody>
</table>

Data were obtained during control conditions (CON) and after adenosine receptor blockade with 8-Phenyloethopine (8-PT; 5 mg/kg i.v.).

*P<0.05 vs CON.
The effects of adenosine on coronary flow are principally mediated by activation of $\alpha_2$ receptors. Blockade of adenosine receptors does not impair the increase in coronary flow during treadmill exercise in the normal heart. Similarly, in anesthetized dogs 8-PT (3 mg/kg iv) failed to block the increase in CBF in response to pacing at a rate that doubled MVO$_2$. In that study, neither coronary venous nor estimated interstitial adenosine concentrations increased to levels sufficient to overcome the adenosine receptor blockade, demonstrating that vasodilator mechanisms other than adenosine are responsible for coronary vasodilation during increases in cardiac work in the normal heart.

Adenosine and the Failing Heart

In the present study nonselective adenosine receptor blockade with 8-PT produced a 26% increase in O$_2$ uptake by the failing heart at rest, which was maintained during exercise. The increase in CBF produced by 8-PT occurred secondary to an increase in myocardial O$_2$ use, with an increase in O$_2$ extraction as evidenced by a significant reduction in coronary venous pO$_2$. However, 8-PT did not alter the relationship between MVO$_2$ and coronary venous pO$_2$, implying that it did not alter metabolic signaling between myocardial myocytes and the coronary resistance vessels.

In dogs with CHF secondary to an infrarenal aortocaval fistula, pericardial adenosine levels were 3 times higher than normal. In humans with CHF, plasma adenosine levels were 3 to 4 times higher than normal and strongly correlated with both plasma norepinephrine and the functional class. Varani et al noted a significant increase in plasma adenosine levels after cardiac transplantation. Because norepinephrine can increase adenosine production from AMP by activation of ecto-5'-nucleotidase, the increased adenosine levels may be mediated by activation of A1-receptor. Taken together, these findings suggest that increased adenosine production in response to AMP by activation of ecto-5'-nucleotidase, the increased adenosine levels may serve to counteract the detrimental effects of norepinephrine.

Adenosine exerts antiadrenergic effects on the myocardi-um by blunting $\beta$-adrenergic stimulation as its binding to the $\alpha_1$-receptor activates the G protein, Gs, which leads to attenuation of adenyl cyclase activity and cAMP production. Because cardiac energy use appears to be downregulated in CHF, whereas adenosine production is increased, it is possible that adenosine may modulate metabolism in the failing heart through antiadrenergic effects on $\alpha_1$ receptors or through inhibition of mitochondrial respiration via NO.

We previously found in dogs with pacing-induced CHF that inhibition of NO production with nitro-l-arginine (LNNA) resulted in increases of MVO$_2$ and CBF that were similar in magnitude to those produced by 8-PT in the present study. NO can inhibit mitochondrial respiration by reversible inhibition at the Fe/Cu centers of cytochrome oxidase. Studies in vascular endothelial cells have demonstrated an increase in NO production in response to adenosine. The vasodilator response of porcine coronary arterioles to adenosine was blunted after endothelial removal or treatment with nitro-l-arginine. In agreement with the concept that NO is released in response to adenosine, further studies are required to determine whether increased adenosine production in the failing heart might stimulate NO production with consequent inhibition of myocardial oxidative metabolism.

Study Limitations

An important consideration is whether 8-PT could have increased MVO$_2$ as the result of the known inhibitory effects of xanthines on phosphodiesterase activity. This effect could potentially increase myocardial cAMP levels, resulting in enhanced contractility and coronary vasodilation. However, aside from a small increase in resting heart rate during resting conditions, no significant change in hemodynamics was observed after 8-PT. The small, nonsignificant increase in dP/dt could be consistent with an increase in ATP available for contraction if adenosine inhibition of MVO$_2$ limits ATP production in the failing heart. Such an effect could be mediated by an adenosine-induced increase in NO production, because intracoronary infusion of NO has been shown to reduce ATP production and contractility. Additionally, phosphodiesterase activity and protein expression are decreased in failing hearts. Alternatively, because circulating catecholamines are increased in this model of CHF, blockade of the adenosine A$_2$ receptor by 8-PT may reduce the normal antiadrenergic effect of adenosine on the myocyte $\beta$-receptor. Taken together, these findings suggest that the increased MVO$_2$ after 8-PT in the failing heart was predominantly attributable to a direct myocardial effect as opposed to PDE inhibition.

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Disclosures

None.

References


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