Glibenclamide Antagonizes Adenosine A₁ Receptor–Mediated Cardioprotection in Stunned Canine Myocardium

Zhenhai Yao, PhD, MD, and Garrett J. Gross, PhD

Background. The main objective of the present study was to determine the role of adenosine in the development of myocardial stunning following multiple, brief periods of coronary artery occlusion as well as the subtype of adenosine receptor (A₁ or A₂) involved. A second objective was to determine if there was an interaction between the adenosine A₁ receptor and the ATP-dependent K channel (K<sub>ATP</sub>).

Methods and Results. The effects of the selective adenosine A₁ receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and agonist cyclopentyladenosine (CPA), the selective A₂ receptor agonist CGS 21680, and the K<sub>ATP</sub> channel blocker glibenclamide on myocardial stunning produced by repetitive coronary artery occlusions were studied in barbital-anesthetized dogs. Regional segment function was measured with sonomicrometry. Under control conditions, six 5-minute periods of coronary occlusion interspersed with 10-minute periods of reperfusion and ultimately followed by 2 hours of reperfusion produced regional segment dysfunction. Pretreatment with intravenous infusion of CPA (2.0 μg·kg⁻¹·min⁻¹) improved percent segment shortening throughout reperfusion, whereas pretreatment with DPCPX (1.0 mg/kg i.v. bolus) significantly worsened the recovery of postischemic contractile function. In contrast, neither DPCPX nor CPA had any effect on the recovery of contractile function when administered before the second coronary occlusion. Furthermore, pretreatment with CGS 21680 (0.2 μg·kg⁻¹·min⁻¹) did not affect the recovery of percent segment shortening. In addition, pretreatment with a low dose of glibenclamide (0.1 mg/kg) had no effect on percent segment shortening by itself but completely abolished the beneficial effect of CPA. Importantly, the effects of the various agents on percent segment shortening were independent of differences in systemic hemodynamics, collateral blood flow, or ischemic bed size.

Conclusions. These results suggest that stimulation of myocardial adenosine A₁ receptors, particularly when induced by the initial coronary artery occlusion, is cardioprotective during repetitive, brief periods of coronary artery occlusion and that these beneficial actions may be partially mediated via a glibenclamide-sensitive mechanism, possibly opening of myocardial K<sub>ATP</sub> channels. (Circulation 1993;88:235–244)

KEY WORDS • occlusions • adenosine • glibenclamide • myocardium

Single or multiple brief periods of coronary artery occlusion interspersed with reperfusion have been shown to produce reversible ischemia-reperfusion injury or myocardial stunning.¹⁻⁷ Although considerable effort has been expended in elucidating the mechanism of stunning following a single coronary occlusion–reperfusion protocol,⁴⁻⁷ less research has been done to elucidate the mechanism of stunning in a multiple-occlusion model. The observation that calcium antagonists improve the recovery of regional contractile function following repetitive episodes of ischemia-reperfusion³⁻⁸ suggests a role for calcium overload during ischemia or early reperfusion, and the finding that superoxide dismutase and catalase improve functional recovery suggests a role for oxygen-derived free radicals.⁹ In addition, disruption of the collagen matrix also has been proposed to be partially responsible for postischemic dysfunction in the multiple-occlusion model.¹⁰

Recently, the protective role of adenosine in myocardial reperfusion injury has received considerable attention.¹¹ Numerous studies have shown that administration of adenosine limits myocardial infarct size and attenuates myocardial stunning in animals subjected to a single coronary artery occlusion.¹²⁻¹⁴ However, the mechanisms by which adenosine exerts its cardioprotective effects and the subtype of adenosine receptor (A₁ or A₂) involved are not fully understood.

Therefore, the main aim of the present study was to determine whether adenosine is involved in the pathogenesis of myocardial stunning produced by multiple coronary artery occlusions and, if so, which subtype of adenosine receptor (A₁ or A₂) is important in its actions. For this purpose, we studied the effects of selective adenosine A₁ and A₂ receptor agonists and an A₁ selective antagonist in anesthetized dogs subjected to repeated coronary artery occlusions.
Finally, Kirsch and coworkers\textsuperscript{15} showed that adenosine A\textsubscript{1} receptors are coupled to K\textsubscript{ATP} channels in rat ventricular myocytes. Previous studies performed in our laboratory also showed that activation of K\textsubscript{ATP} channels attenuates and blockade of these channels exacerbates stunning in a multiple-occlusion model.\textsuperscript{10} Therefore, a second objective of this study was to determine whether adenosine A\textsubscript{1} receptor-mediated cardioprotection was mediated via the K\textsubscript{ATP} channel. To test this hypothesis, we studied the effect of a K\textsubscript{ATP} channel antagonist, glibenclamide, on the beneficial action of adenosine A\textsubscript{1} receptor stimulation.

Methods

General Preparation of Dogs

All experiments conducted in this study were in accordance with the "Position of the American Heart Association on Research and Animal Use" adopted in 1984 by the American Heart Association and the guidelines of the Animal Care Committee of the Medical College of Wisconsin. The Medical College of Wisconsin is accredited by the American Association of Laboratory Animal Care.

Adult mongrel dogs of either sex (19.0 to 29.0 kg) were fasted overnight, anesthetized with intravenous administration of sodium barbital (200 mg/kg) and sodium pentobarbital (15 mg/kg), and ventilated with a respirator (model 607, Harvard Apparatus, South Natick, Mass) with room air supplemented with 100% oxygen at an end-expiratory pressure of 5 to 7 cm H\textsubscript{2}O to prevent atelectasis. A double-pressure transducer-tipped catheter (PC 771, Millar Instruments, Houston, Tex) was inserted into the aorta and left ventricle via the left carotid artery for measurement of aortic and left ventricular pressures. Left ventricular dP/dt was determined by electronic differentiation of the left ventricular pressure pulse.

A left thoracotomy was performed at the fifth intercostal space, the lung was retracted, the pericardium was incised, and the heart was suspended in a cradle. A 1.0- to 1.5-cm segment of the left anterior descending coronary artery (LAD) was dissected from surrounding tissue distal to the first diagonal branch, and a calibrated electromagnetic flow probe (Statham SP 7515) was placed around the vessel. A flowmeter (Statham 2202) was used to measure coronary blood flow, and a micrometer-driven mechanical occluder was placed distal to the flow probe. The occluder was used to zero the flow probe (LAD was occluded for 10 seconds) 20 minutes before the initial coronary occlusion and later to occlude the artery. If the basal heart rate was less than 150 beats per minute, the heart was paced at that rate with rectangular pulses of 4-ms duration and a voltage twice threshold via bipolar electrodes sutured to the left atrial appendage. Pacing was not used in the few animals with initial rates of more than 150 beats per minute. Heart rate, hemodynamics, and LAD blood flow were monitored and recorded with a polygraph (model 7, Grass Instrument Co., Quincy, Mass) throughout the experiment. The left atrial appendage was cannulated for radioactive microsphere injection, and the right femoral artery was cannulated for withdrawal of a reference blood flow sample used to measure myocardial tissue blood flow. The right femoral vein was cannulated for drug administration.

Arterial blood pH, P\textsubscript{O\textsubscript{2}}, and P\textsubscript{CO\textsubscript{2}} were monitored at selected intervals with an automatic blood gas system (AVL 995, AVL Scientific Corporation, Roswell, Ga), and maintained within a normal physiological range (pH 7.35 to 7.45; and P\textsubscript{O\textsubscript{2}}, 80 to 120 mm Hg; P\textsubscript{CO\textsubscript{2}}, 25 to 40 mm Hg) by adjusting the respiratory rate and oxygen flow and by intravenous infusion of 1.5% sodium bicarbonate when necessary. Body temperature was maintained at 38±1°C by using a heating pad.

Chemicals and Preparations

Cyclopentyladenosine (CPA), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), and 2-[p-(2-carboxyethyl)-phenethylaminio]-5'-N-ethylcarboxamidoadenosine (CGS 21680) were purchased from Research Biochemicals Inc., Natick, Mass. Glibenclamide was purchased from Sigma Chemical. All solutions were prepared on the day of the experiment. The dose of DPCPX, CPA, or CGS 21680 used for each dog was dissolved in 2 mL polyethylene glycol and 4 mL 0.1 N NaOH, and the dose of glibenclamide for each dog was dissolved in 0.5 mL propylene glycol, 0.5 mL ethanol, and 0.5 mL 1 N NaOH.

Experimental Protocol

Dogs were assigned randomly to one of eight series: 1) vehicle control, 2) DPCPX, 3) CPA, 4) CGS 21680, 5) GLIB, 6) GLIB+CPA, 7) CPA given at 2 minutes before the second occlusion, 8) DPCPX given at 2 minutes before the second occlusion, or 9) DPCPX given at 1 hour after the sixth occlusion. A schematic of the experimental protocol for this study is given in Fig 1. All animals were subjected to six 5-minute periods of LAD occlusion interspersed by 10-minute periods of reperfusion and ultimately followed by 2 hours of reperfusion. Segment shortening (percent) and hemodynamics were recorded throughout the experiment, and regional myocardial blood flow was measured during the first and the sixth occlusion periods and at the end of 2 hours of reperfusion.

Of vehicle-treated control dogs, three received the vehicle for dissolving glibenclamide (0.5 mL propylene glycol, 0.5 mL ethanol, and 0.5 mL 1 N NaOH), and four received the vehicle for dissolving the adenosine agonists and antagonists (2 mL polyethylene glycol and 4 mL 0.1 N NaOH). Because there were no differences between the two vehicle-treated groups regarding hemodynamics, blood flow, ischemic bed size, or percent segment shortening, these data were pooled into one control group (series 1). The effects of blockade of adenosine A\textsubscript{1} receptors on regional contractile dysfunction produced by repetitive coronary artery occlusions were studied by administration of DPCPX (1.0 mg/kg i.v. bolus), a selective adenosine A\textsubscript{1} receptor antagonist,\textsuperscript{17} 15 minutes before the first occlusion period (series 2), 2 minutes before the second occlusion period (series 7), and 1 hour after the sixth occlusion period (series 8), whereas the effect of activation of A\textsubscript{1} receptors was studied by intravenous infusion of CPA (2.0 \mu g \cdot kg\textsuperscript{-1} \cdot min\textsuperscript{-1}, series 3), a selective adenosine A\textsubscript{1} receptor agonist,\textsuperscript{18} starting 15 minutes before the first occlusion period and continuing throughout the sixth occlusion period. The effect of activating adenosine A\textsubscript{2}
receptors (series 4) was studied by intravenous infusion of CGS 21680 (0.2 μg · kg⁻¹ · min⁻¹), a selective adenosine A₂ receptor agonist, starting 15 minutes before the first occlusion period and continuing throughout the sixth occlusion period. Doses of CPA and CGS 21680 that produced an approximately 5-mL/min increase in coronary blood flow without evidence of systemic hypotension were chosen based on results obtained in preliminary dose-response studies and previous work of Norton and coworkers. Animals in series 5 received glibenclamide (0.1 mg/kg iv bolus), a specific K<sub>ATP</sub> channel blocker, 15 minutes before the initial occlusion period, and animals in series 6 received both glibenclamide and CPA. Effects of the compounds on percent segment shortening in the ischemic region were compared with the vehicle-treated control group (series 1).

**Ischemic Area and Regional Myocardial Blood Flow**

Ischemic area size was determined by injecting India ink into the LAD at the site of the occlusion at the end of the experiments and measuring regional myocardial tissue blood flow of the normal and ischemic regions with the radioactive microsphere technique as described previously in this laboratory. Carbonized plastic microspheres (15 μm diameter, New England Nuclear, Boston, Mass) labeled with ⁱ⁴C₁₄, ¹⁰⁸Ru, or ⁹⁵Nb were suspended in isotonic saline with 0.01% Tween 80 added to prevent aggregation. The microspheres were ultrasonicated for 5 minutes and vortexed for another 5 minutes before injection. One milliliter of the microsphere suspension (2 to 4×10⁴ microspheres) was given via the left atrial catheter and flushed by 5 mL of saline. A reference blood flow sample was drawn from the femoral artery at a constant rate of 7.1 mL/min immediately before microsphere injection. Regional myocardial tissue blood flow was measured at 2 minutes during the first and sixth occlusion periods as well as at the end of the final reperfusion period.

On the following day, tissue slices were sectioned into subepicardium, midmyocardium, and subendocardium of nonischemic (three pieces) and ischemic regions (five pieces). The tissue samples were weighed (0.3 to 0.8 g). Transmural pieces were obtained from the center of several transverse sections used to determine the ischemic area size and were at least 1 cm from the perfusion boundaries as indicated by patent blue dye. All samples were counted in a gamma counter (Tracer Analytic 1195, TM Analytic, Elk Grove, III) to determine the activity of each isotope in each sample. The activity of each isotope also was determined in the reference blood flow samples. Myocardial blood flow was calculated by using a preprogrammed computer (Apple IIe) to obtain the true activity of each isotope in individual samples, and tissue blood flow was calculated from the equation

\[ Q_m = Q_r \times C_m / C_r \]

where \( Q_m \) is myocardial blood flow (mL · min⁻¹ · g⁻¹), \( Q_r \) is the rate of withdrawal of the reference blood flow (7.1 mL/min), \( C_r \) is the activity of the reference blood flow sample (counts per minute [cpm]), and \( C_m \) is the activity of the tissue sample (cpm/g).
Myocardial Segment Shortening

Myocardial segment shortening in the ischemic and nonischemic regions was assessed by two sets of piezoelectric crystals inserted 7 to 9 mm into the subendocardium. An ultrasonic amplifier was connected to the leads of the crystals. The amplifier transforms the sound pulses from the crystals into an electrical signal that is proportional to the distance between them. The tracings were monitored with an oscilloscope (Soltec model 520), and the distance between the two crystals was measured by changes in transmission time and recorded on a polygraph. Segment length during diastole (DL) and systole (SL) was determined at the onset of isovolumetric contraction (beginning of positive dP/dt) and at peak negative dP/dt, respectively. Percent segment shortening (%SS) was calculated from the equation: \%SS = (DL - SL)/DL × 100. The segment length data were normalized by using a value of 10.0 for the baseline DL.

Criteria for Exclusions

Dogs were excluded if subendocardial collateral flow was more than 0.15 mL·min⁻¹·g⁻¹, ventricular fibrillation occurred, heart rate was more than 160 beats per minute, or heartworms were found after dogs were killed.

Statistical Analysis

All values are given as the mean±SEM. A two-way ANOVA with Fisher's least significant difference was used to compare differences between groups, and a one-way ANOVA with Dunnett's t test was used to compare differences within groups. P<.05 was considered significantly different.

Results

Exclusions

A total of 63 dogs were used in this study. Six dogs were excluded because subendocardial blood flow was more than 0.15 mL·min⁻¹·g⁻¹ (one each in series 1, 3, 7, and 8; two in series 2), another four were excluded because of ventricular fibrillation (two in series 1, one each in series 4 and 6), and another in series 5 was excluded because heartworms were found after the heart was removed. Thus, 52 dogs were used in data analysis (n=7 for series 1 and 2; n=6 for series 3, 4, 5, 6, and 8; n=5 for series 7; and n=3 for series 9).

Body Weight, Ischemic Area, and Infarct Size

Body weight, left ventricular weight, and ischemic area weight for all animals used in this study were 22.1±0.3 kg, 105.6±2.2 g, and 28.3±0.8 g, respectively, and no significant differences were found between groups. There was no evidence for infarcted tissue in any of the animals studied by TTC staining.

Hemodynamics and Blood Gases

Heart rate, mean aortic blood pressure, the rate-pressure product, left ventricular dP/dt, and LAD blood flow were monitored throughout the experiment, and the baseline values for all series are summarized in Table 1. Hemodynamics were not significantly affected by drug treatment except that heart rate at 2 hours of reperfusion in groups pretreated with glibenclamide and the combination of glibenclamide plus CPA was slightly higher than that observed in controls. There were no significant differences in baseline values for blood gases and pH between groups (Table 2), and no statistically significant changes occurred throughout the experiment.

Regional Myocardial Blood Flow

Regional myocardial blood flow data in the ischemic (LAD) and nonischemic (left circumflex) regions are shown in Table 3. There were no significant differences between groups in blood flow to any layers of the ischemic myocardium. These data indicate that all groups of animals were subjected to equal degrees of

### Table 1. Baseline Values of Systemic Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (bpm)</th>
<th>MAP (mm Hg)</th>
<th>RPP (mm Hg·min⁻¹·1000⁻¹)</th>
<th>LV dP/dt (mm Hg/s)</th>
<th>CBF (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>150±1</td>
<td>105±5</td>
<td>17.7±1.1</td>
<td>1903±208</td>
<td>35±4</td>
</tr>
<tr>
<td>DPCPX pretreatment</td>
<td>152±2</td>
<td>113±7</td>
<td>19.5±1.4</td>
<td>2304±192</td>
<td>36±4</td>
</tr>
<tr>
<td>DPCPX before second occlusion</td>
<td>148±4</td>
<td>98±6</td>
<td>16.5±1.4</td>
<td>2050±250</td>
<td>32±9</td>
</tr>
<tr>
<td>CPA pretreatment</td>
<td>153±2</td>
<td>114±6</td>
<td>19.7±1.2</td>
<td>1950±263</td>
<td>37±5</td>
</tr>
<tr>
<td>CPA before second occlusion</td>
<td>153±3</td>
<td>93±7</td>
<td>16.2±1.3</td>
<td>1860±240</td>
<td>34±3</td>
</tr>
<tr>
<td>CGS 21680 pretreatment</td>
<td>153±2</td>
<td>108±8</td>
<td>18.4±1.7</td>
<td>1875±297</td>
<td>35±5</td>
</tr>
<tr>
<td>GLIB pretreatment</td>
<td>154±3</td>
<td>110±8</td>
<td>18.9±1.7</td>
<td>2050±354</td>
<td>45±6</td>
</tr>
<tr>
<td>GLIB+CPA pretreatment</td>
<td>157±4</td>
<td>104±3</td>
<td>18.4±0.6</td>
<td>2225±224</td>
<td>31±8</td>
</tr>
</tbody>
</table>

MAP, mean aortic pressure; RPP, rate-pressure product; LV, left ventricular; CBF, coronary blood flow; CPA, cyclopentyladenosine; GLIB, glibenclamide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine. Values are given as mean±SEM. There were no significant differences among groups.

### Table 2. Baseline Values of Blood Gases and pH

<table>
<thead>
<tr>
<th></th>
<th>PO₂ (mm Hg)</th>
<th>PCO₂ (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>110±4</td>
<td>33±2</td>
<td>7.41±0.02</td>
</tr>
<tr>
<td>DPCPX pretreatment</td>
<td>103±6</td>
<td>32±2</td>
<td>7.42±0.02</td>
</tr>
<tr>
<td>DPCPX before second occlusion</td>
<td>101±6</td>
<td>34±2</td>
<td>7.40±0.01</td>
</tr>
<tr>
<td>CPA pretreatment</td>
<td>100±6</td>
<td>37±2</td>
<td>7.39±0.02</td>
</tr>
<tr>
<td>CPA before second occlusion</td>
<td>99±4</td>
<td>30±2</td>
<td>7.41±0.03</td>
</tr>
<tr>
<td>CGS 21680 pretreatment</td>
<td>102±7</td>
<td>32±3</td>
<td>7.41±0.03</td>
</tr>
<tr>
<td>GLIB pretreatment</td>
<td>102±7</td>
<td>33±2</td>
<td>7.40±0.02</td>
</tr>
<tr>
<td>GLIB+CPA pretreatment</td>
<td>103±8</td>
<td>37±2</td>
<td>7.37±0.02</td>
</tr>
</tbody>
</table>

CPA, cyclopentyladenosine; GLIB, glibenclamide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine. Values are given as mean±SEM. There were no significant differences among groups.
ischemia throughout the six occlusion periods. In the nonischemic region, blood flow was significantly lower in the glibenclamide-pretreated group, the CPA-pretreated group, and the group in which DPCPX was given after the first occlusion compared with that in the control group.

**Segment Function**

Six 5-minute periods of coronary artery occlusion interspersed by 10-minute periods of reperfusion and finally followed by 2 hours of reperfusion produced a marked loss of myocardial segment function throughout each reperfusion period compared with the baseline control value (Figs 2 to 4 control series). Pretreatment with DPCPX dramatically worsened the recovery of percent segment shortening throughout each reperfusion period compared with the control series (Fig 2A). However, no effects on stunning were observed when DPCPX was given 2 minutes before the second occlusion period (Fig 2B) or 1 hour after the sixth occlusion. On the other hand, pretreatment with CPA significantly attenuated myocardial stunning during all periods of reperfusion (Fig 3A); however, CPA had no effect on stunning when given 2 minutes before the second occlusion (Fig 3B). In addition, pretreatment with CGS 21680 had no effect on segment function (data not shown). Finally, glibenclamide by itself did not affect percent segment shortening (Fig 4A); however, it completely abolished the beneficial effect of CPA (Fig 4B) on contractile function. Percent segment shortening in the nonischemic area was not statistically different between series throughout the experiments (data not shown).

**TABLE 3. Regional Myocardial Blood Flow**

<table>
<thead>
<tr>
<th></th>
<th>Nonischemic region (left circumflex coronary artery) (mL·min⁻¹·g⁻¹)</th>
<th>Ischemic region (left anterior descending coronary artery) (mL·min⁻¹·g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Occ1</td>
<td>Occ6</td>
</tr>
<tr>
<td>Epicardium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.20±0.13</td>
<td>1.10±0.13</td>
</tr>
<tr>
<td>DPCPX pretreatment</td>
<td>1.40±0.07</td>
<td>1.10±0.19</td>
</tr>
<tr>
<td>DPCPX before second occlusion</td>
<td>0.77±0.08*</td>
<td>0.66±0.14*</td>
</tr>
<tr>
<td>CPA pretreatment</td>
<td>0.93±0.17</td>
<td>0.82±0.13</td>
</tr>
<tr>
<td>CPA before second occlusion</td>
<td>1.00±0.11</td>
<td>1.00±0.22</td>
</tr>
<tr>
<td>CGS 21680 pretreatment</td>
<td>1.07±0.18</td>
<td>1.14±0.28</td>
</tr>
<tr>
<td>GLIB pretreatment</td>
<td>0.73±0.09*</td>
<td>0.79±0.12*</td>
</tr>
<tr>
<td>GLIB+CPA pretreatment</td>
<td>1.20±0.21</td>
<td>1.38±0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midmyocardium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.35±0.16</td>
<td>1.23±0.14</td>
</tr>
<tr>
<td>DPCPX pretreatment</td>
<td>1.63±0.28</td>
<td>1.17±0.18</td>
</tr>
<tr>
<td>DPCPX before second occlusion</td>
<td>0.92±0.09*</td>
<td>0.77±0.17*</td>
</tr>
<tr>
<td>CPA pretreatment</td>
<td>1.05±0.17</td>
<td>0.84±0.13*</td>
</tr>
<tr>
<td>CPA before second occlusion</td>
<td>1.10±0.16</td>
<td>1.04±0.20</td>
</tr>
<tr>
<td>CGS 21680 pretreatment</td>
<td>1.33±0.25</td>
<td>1.30±0.31</td>
</tr>
<tr>
<td>GLIB pretreatment</td>
<td>0.80±0.12*</td>
<td>0.77±0.08*</td>
</tr>
<tr>
<td>GLIB+CPA pretreatment</td>
<td>1.32±0.22</td>
<td>1.44±0.26</td>
</tr>
</tbody>
</table>

CPA, cyclopentyladenosine; GLIB, glibenclamide; Occ1 and Occ6, during occlusion periods 1 and 6; Rep (2 h), at 2 hours after the sixth occlusion.

Values are given as mean±SEM. *Significantly different from the control series.
emic contractile dysfunction, and 3) the protective effects of adenosine A₁ receptor activation were completely antagonized by glibenclamide, a K<sub>ATP</sub> channel blocker.

Initially, we observed that six brief, consecutive ischemia-reperfusion cycles produced regional contractile dysfunction primarily following the first occlusion period but to a much lesser extent following the subsequent five occlusion periods. These results agree with the findings of other investigators who also have shown that the largest decrease in contractile function occurs after the initial occlusion period and that additional decrements in contractility appeared to be progressively smaller. These observations suggest that an endogenous adaptive mechanism(s) may exist in the heart that is turned on during the first brief occlusion period. Adenosine, a metabolite of adenine nucleotides, is released from the heart during ischemia, and activation of adenosine A<sub>1</sub> or A<sub>2</sub> receptors has been shown to ameliorate irreversible ischemia-reperfusion injury in an infarct model. More recently, Bunch et al proposed that endogenously released adenosine acted to preserve wall motion in a repetitive coronary artery occlusion model. Thus, it seems reasonable to hypothesize that endogenous adenosine released during the initial coronary occlusion period may activate adenosine A<sub>1</sub> receptors in the ischemic myocardium, thus rendering the myocardium resistant to subsequent ischemic insults. That the decrease in percent segment shortening during reperfusion following occlusion periods 2 to 6 was much less than that observed after occlusion period 1 and that either DPCPX or CPA given before occlusion period 2 had no effect on the recovery of percent segment shortening supports this hypothesis.

Evidence that the A<sub>1</sub> receptor mediates the protective effect of adenosine in this model was obtained by the use of DPCPX, a xanthine derivative that has been demonstrated to be 700-fold selective for the adenosine A<sub>1</sub> receptor versus the A<sub>2</sub> receptor using radioligand binding techniques and in vitro functional assays. We found that DPCPX worsened the recovery of myocardial segment function throughout reperfusion in the
absence of systemic hemodynamic effects, whereas the compound had no effect when given before occlusion period 2 or 1 hour after the sixth occlusion period. These data suggest that DPCPX exacerbates myocardial stunning by blocking the effects of adenosine during the first occlusion period and that the adenosine $A_2$ receptor is important in the pathogenesis of myocardial stunning in this model. Second, we found that pretreatment with CPA, a potent and selective adenosine $A_2$ receptor agonist, significantly improved the recovery of regional contractile function without significantly affecting coronary blood flow and mean aortic blood pressure. However, CPA, similar to DPCPX, had no effects on stunning when administered before the second occlusion. These data suggest that CPA exerts its cardioprotective action primarily during the initial, brief period of myocardial ischemia through a direct effect on adenosine $A_2$ receptors in myocytes. A direct myocardial effect mediated by $A_2$ receptor activation was not observed since CGS 21680, a selective adenosine $A_2$ receptor agonist, did not affect the recovery of contractile function at the dose studied in this model.

However, it is still possible that $A_2$ receptor stimulation by a higher dose of CGS 21680 would produce a beneficial effect via its potent coronary vasodilating activity or an effect to inhibit neutrophil function. More recently, Lasley and Mentzer, using isolated rat hearts, demonstrated that adenosine enhanced postischemic myocardial function via activation of the adenosine $A_1$ receptor but not the $A_2$ receptor. Taken together, these results imply that adenosine $A_2$ receptor activation, by either endogenous adenosine or exogenous CPA, is an important mechanism for preservation of myocardial contractile function. Stimulation of adenosine $A_2$ receptors also has been shown in several myocardial infarct studies to reduce ischemia-reperfusion damage and to be an important contributory factor in ischemic preconditioning, a phenomenon known to markedly limit infarct size.

The mechanisms by which activation of adenosine $A_2$ receptors protects the heart against ischemia-reperfusion injury remain to be defined. It is known that large amount of endogenous catecholamines are released during myocardial ischemia. Activation of adenosine
A1 receptors has been shown to reduce catecholamine release.39 Activation of adenosine A1 receptors also has been shown to result in an increase in glucose influx in isolated, perfused rat hearts and an increase in myocardial ATP production.34 Furthermore, adenosine has been shown to decrease intracellular ATP loss, decrease intracellular cAMP levels,35 and produce a negative inotropic effect.36 Thus, multiple effects resulting from adenosine A1 receptor stimulation may be at least partially responsible for the beneficial actions of CPA.

Numerous studies with calcium antagonists indicate a role for calcium overload in myocardial stunning.3,8,37,38 Previous studies performed in our laboratory demonstrated that amlodipine, a calcium antagonist, improved myocardial functional and metabolic recovery of stunned myocardium in a multiple-occlusion model.8 Recently, Gross and coinvestigators9 demonstrated that oxygen-derived free radicals were involved in the pathogenesis of reperfusion injury in a multiple-coronary-occlusion model.9 Activation of A1 receptors also has been proposed21 to decrease free radical formation during reperfusion by reducing lipolysis, thus inhibiting the formation of lipid hydroperoxides, and by decreasing the quantity of catecholamines available for autoperoxidation. Thus, adenosine or stimulation of A1 receptors may act to inhibit calcium overload and/or reduce oxygen-derived free radical–induced damage in this model.

Another possible mechanism by which activation of adenosine A1 receptors protects the ischemic myocardium is via opening KATP channels. Adenosine has been shown to affect the activity of various ion channels in both atrial and ventricular myocytes.39 That stimulation of adenosine A1 receptors leads to opening of myocardial KATP channels originally was demonstrated in rat ventricular myocytes by Kirsch et al.15 This concept has been confirmed further in the canine heart.40 Recently, we showed that pretreatment with 0.3 mg/kg glibenclamide, a specific KATP channel antagonist,16 significantly worsened and that pretreatment with aprikalim, a KATP channel opener,42 markedly improved the recovery of percent segment shortening in stunned myocardium.16

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**Fig 4.** Plots of percent segment shortening in the ischemic region for the vehicle-treated control group (series 1) (○–○). The absolute baseline values for segment shortening ([diastolic length minus systolic length]/diastolic length×100) for series 1, 5, and 6 were 19.4±1.8%, 22.4±2.5%, and 22.7±2.9%, respectively, and there were no statistical differences among groups. Panel A: Percent segment shortening in the ischemic region for the glibenclamide (GLIB) pretreatment group (series 5). Panel B: Percent segment shortening in the ischemic region for the GLIB+cyclopentyladenosine (CPA) treatment group (series 6). All values are mean±SEM. There were no significant differences between the drug-treated and the vehicle-treated groups.
An interesting finding in the current study was that pretreatment with a low dose of glibenclamide (0.1 mg/kg) that had no effect on percent segment shortening by itself completely abolished the protective effect of CPA. Although it might be expected that 0.1 mg/kg glibenclamide alone would worsen the recovery of percent segment shortening in a manner similar to DPCPX by blocking the beneficial effect of endogenous adenosine, this was not the case. The reason for the lack of effect of this low dose of glibenclamide is not clear; however, because it usually is more difficult to block the effects of endogenously released hormones and neurotransmitters than exogenously administered substances, it seems likely that the low dose of glibenclamide was just above some critical threshold that was effective only at antagonizing the effects of exogenously administered CPA. Nevertheless, because the protective effect of adenosine A1 receptor activation was prevented by glibenclamide, there is in vivo evidence that adenosine A1 receptors interact with KATP channels in the canine myocardium. These results are in agreement with recent results from our laboratory in which it was found that glibenclamide antagonized the infarct-reducing effect of intracoronary adenosine.43 Obviously, further studies are needed to elucidate the mechanism by which adenosine A1 receptors interact with KATP channels to protect the ischemic myocardium.

In conclusion, the present study suggests that 1) endogenously released adenosine, particularly during the initial brief period of coronary artery occlusion, plays a crucial role in the pathogenesis of myocardial stunning and may be an important endogenous mechanism that provides a cardioprotective effect during repetitive, brief episodes of myocardial ischemia; 2) the beneficial actions of adenosine are mediated via adenosine A1 receptor activation; and 3) an interaction between adenosine A1 receptor activation and a glibenclamide-sensitive mechanism exists in the canine myocardium. That an endogenous protective mechanism is linked to stimulation of adenosine A1 receptors in the heart suggests that the development of pharmacological agents that act selectively on this receptor may have great promise as myocardial protective agents for clinical applications.

Acknowledgments

The authors wish to express their sincere appreciation to Anna Hsu and Jeannine Moore for their artwork and excellent technical assistance in the completion of this project and the preparation of the manuscript.

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Z Yao and G J Gross

Circulation. 1993;88:235-244
doi: 10.1161/01.CIR.88.1.235
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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