Role of Myocardial ATP-Sensitive Potassium Channels in Mediating Preconditioning in the Dog Heart and Their Possible Interaction With Adenosine A<sub>1</sub>-Receptors

Gary J. Grover, PhD; Paul G. Sleph, BS; and Steven Dzwonczyk, BS

**Background.** A brief period of myocardial ischemia can result in an increased resistance to subsequent, more severe episodes of ischemia. Recent studies have indicated that activation of adenosine A<sub>1</sub>-receptors may mediate this preconditioning effect. It is also known that A<sub>1</sub>-activation can lead to ATP-sensitive potassium channel (K<sub>ATP</sub>) opening via a G<sub>i</sub> protein–mediated effect. Thus, we determined whether the K<sub>ATP</sub> blocker glyburide could abolish preconditioning or the protective effects of A<sub>1</sub>-receptor activation.

**Methods and Results.** Anesthetized dogs were subjected to 5 minutes of left circumflex coronary artery (LCx) occlusion (or sham) followed by 10 minutes of reperfusion. The hearts were then subjected to 60 minutes of LCx occlusion and 5 hours of reperfusion. Glyburide (5 μg/kg/min) or vehicle was given directly into the LCx 20 minutes before preconditioning or sham preconditioning. Preconditioning resulted in a significantly reduced infarct size compared with nonpreconditioned animals. Glyburide abolished the protective effect of preconditioning. To establish a link between K<sub>ATP</sub> and A<sub>1</sub>-receptor activation, the effect of the A<sub>1</sub>-agonist R-PIA with or without glyburide on infarct size was determined. R-PIA (0.4 μg/kg/min, directly into the LCx) significantly reduced infarct size, and this protective effect was abolished by glyburide. None of the treatments described above had a significant effect on peripheral hemodynamic status or myocardial blood flow.

**Conclusions.** Preconditioning may be mediated by K<sub>ATP</sub> activation, and this may be linked to A<sub>1</sub>-receptor stimulation. *(Circulation 1992;86:1310–1316)*

**Key Words** • ischemia, myocardial • reperfusion • glibenclamide • infarction

Brief periods of ischemia can render the heart more resistant to subsequent, more severe episodes of ischemia. The protective effects of myocardial preconditioning have been demonstrated in several mammalian species including dogs, rabbits, and pigs. Investigations are currently ongoing to determine the mechanism of preconditioning. Several investigators have shown that preconditioning does not induce an increased collateral blood flow into the ischemic region. Several studies have indicated that adenosine may be mediating preconditioning, apparently via A<sub>1</sub>-receptor activation. Selective A<sub>1</sub>-receptor antagonists have been shown to abolish preconditioning, and A<sub>1</sub>-agonists appear to mimic preconditioning. The mechanism in which A<sub>1</sub>-activation can protect ischemic tissue is unknown, although some evidence indicates an involvement of a G<sub>i</sub> protein pathway.

Recent evidence has indicated a link between adenosine receptors and myocardial ATP-sensitive potassium channels (K<sub>ATP</sub>). In myocytes, A<sub>1</sub>-receptor activation can open K<sub>ATP</sub> via a G<sub>i</sub> protein–mediated process. In agreement with these data are several reports indicating that coronary reactive hyperemia and adenosine-induced increases in coronary blood flow can be attenuated by the K<sub>ATP</sub> blocker glyburide (glibenclamide). Since several laboratories have shown that pharmacological activation of K<sub>ATP</sub> can protect ischemic myocardial tissue in several species, this may explain how activation of A<sub>1</sub>-receptors can protect ischemic myocardium. With this in mind, Gross and associates found that the structurally dissimilar K<sub>ATP</sub> blockers glyburide and sodium 5-hydroxydecanoate abolished preconditioning in dogs. Unfortunately, work from another group has shown glyburide to be ineffective in abolishing preconditioning in rabbits. Thus, the role of K<sub>ATP</sub> activation in mediating preconditioning is still debatable. The purpose of the present study was to determine whether glyburide can abolish preconditioning in a canine model of ischemia and reperfusion. In addition, we also determined whether the cardioprotective effects of A<sub>1</sub>-stimulation can be blocked by glyburide. The results of this study will further define the role of K<sub>ATP</sub> activation in preconditioning.

**Methods**

**Surgical Preparation**

Mongrel dogs of either sex (weight, 10–17 kg) were anesthetized with intravenous sodium pentobarbital (30 mg/kg), and a catheter was placed into the right femoral
artery for collection of blood samples. A Millar Mikrotip catheter pressure transducer (Millar Instruments Inc., Houston, Tex.) was placed into the left femoral artery and advanced into the aortic arch for the measurement of arterial blood pressure. An endotracheal tube was placed into the trachea, and the animals were artificially respirated such that eucapnia was maintained. Eucapnia ($P_CO_2$ 35–40 mm Hg) and normoxia (>70 mm Hg) were maintained throughout the experiment by continuous monitoring of arterial blood gases using an ABL 4 blood gas analyzer (Radiometer, Copenhagen).

A left thoracotomy was performed at the fifth intercostal space, and the heart was exposed. The left circumflex coronary artery (LCx) was isolated proximal to its first branch, and a silk suture was placed around it for later occlusion. A catheter was placed into the left atrial appendage for dye and radioactive microsphere injection. A catheter was also placed into the L CX distal to the coronary snare, and this was to be used for intracoronary infusion of drug or vehicle. The catheter consisted of a bent 27-gauge needle with polyethylene tubing attached.

The animals were allowed to stabilize for 5–10 minutes, at which time an arterial blood sample was removed anaerobically for measurement of blood gases. Arterial blood pressure, heart rate, and ECG were measured. At this time, myocardial blood flow was measured using radioactive microspheres ($^{149}$Sn, $^{57}$Co, $^{85}$Sr, or $^{86}$Sc; 15±3 μm; New England Nuclear Company, Wilmington, Del.). Animals that were to be preconditioned were subjected to a 5-minute LCx occlusion followed by 10 minutes of reperfusion. The LCx was then completely occluded for a total of 60 minutes in all groups. Myocardial blood flow was determined 40 minutes after the initiation of the second LCx occlusion. At 60 minutes after occlusion, the LCx was reperfused. After 1 hour of reperfusion, myocardial blood flow was again measured.

The reperfusion was continued for a total of 5 hours, at which time the LCx was cannulated and perfused at the animal's existing pressure with Ringer's lactate for determination of the area at risk. Patent blue dye (1 mg/kg of a 10 mg/ml solution) was injected into the left atrial catheter, and the heart was quickly excised. The atria were trimmed away, leaving only the ventricles. The ventricles were then cut transversely into 0.5-cm slices. The borders of the area at risk (no dye) were delineated and separated, and the slices were incubated at 37°C for 30 minutes in a 1% solution of 2,3,5-triphenyl tetrazolium chloride in phosphate-buffered saline. This dye stains viable tissue red, whereas infarcted tissue is not stained and becomes white or gray in color. The ventricular slices were dried, and both sides were carefully traced onto clear transparencies. The area at risk and the infarcted region were demarcated on the transparencies. These tracings were transferred to paper, and the areas of interest were measured using planimetric techniques. The infarct size was expressed as a percent of the left ventricular (LV) area at risk. Myocardial blood flow was calculated by taking pieces from the subepicardial and subendocardial halves of the ischemic and nonischemic regions of the left ventricular free wall. The radioactivity in the tissue pieces as well as the reference blood samples were determined in a Beckman Autogamma 8000 gamma counter, and tissue flows were calculated from these counts.

### Drug Treatment

In the first study, the effect of the $K_{ATP}$ blocker glyburide on preconditioning was determined. The animals were divided into four groups: group 1, animals given vehicle (50% polyethylene glycol in water, $n=7$) without preconditioning (sham); group 2, animals given vehicle before preconditioning ($n=8$); group 3, animals given 5 μg/kg/min glyburide (Sigma Chemical Co., St. Louis, Mo.) without preconditioning ($n=6$); and group 4, animals given 5 μg/kg/min glyburide before preconditioning ($n=9$). The glyburide or vehicle was administered directly into the LCx. The glyburide or vehicle was given starting 5 minutes before preconditioning or sham (groups 1 and 3 were not preconditioned) and continued for a total of 20 minutes (infusion stopped immediately before the second, more severe LCx occlusion). The dose of glyburide used was found to completely abolish the coronary dilator effect of 1 μg/kg/min infusion of cromakalim ($K_{ATP}$ opener) in dogs (data not shown; similar results have been previously shown by Auchampach et al. for a slightly lower dose of glyburide. This dose of cromakalim causes a 35% increase in coronary flow in this preparation. This glyburide protocol was found to have no effect on plasma glucose concentrations, which is not surprising because of the local intracoronary infusion of this agent. After the preconditioning or drug treatment, the LCx was occluded for 60 minutes and reperfused for 5 hours as described above. Total volume administered for vehicle and glyburide solutions was 10 ml.

In another group of animals, the interaction between $A_1$-receptors and $K_{ATP}$ in the above infarction model was determined. The animals were divided into three groups: group 1, animals given vehicle (50% polyethylene glycol in water, $n=7$); group 2, animals given 0.4 μg/kg/min R-PIA ($R$-N-(2-phenylisopropyl) adenosine, Research Biochemicals Inc., Natick, Mass.) ($n=7$); and group 3, animals given 5 μg/kg/min glyburide+0.4 μg/kg/min R-PIA ($n=6$). The various treatments were administered directly into the LCx. None of the animals in this group were subjected to preconditioning, although a similar time course was allowed as for preconditioned animals. The infusion of R-PIA was given as an infusion over 15 minutes before LCx occlusion, and glyburide was infused over 20 minutes before LCx occlusion. Thus, glyburide infusion was begun 5 minutes before R-PIA infusion. After drug treatment, the animals were subjected to 60 minutes of LCx occlusion and 5 hours of reperfusion, as described above. The dose of R-PIA was selected as the highest dose in which no heart rate effects were observed. As above, the total volume of vehicle and drug groups was the same with the animals receiving a total volume of 10 ml.

### Statistics

Changes in hemodynamic, blood flow, and infarct size variables were analyzed using ANOVA. Multiple comparisons were done using the Newman–Keuls test. Linear regression analysis was used to evaluate the relation between LV area at risk versus LV infarct size and LV infarct size as a percent of area at risk versus subendocardial collateral blood flow. ANCOVA was used to determine differences in these relations for the various drug treatments. This form of infarct size analysis has been previously described in detail. For all analyses,
The effect of glyburide and preconditioning on myocardial infarct size and LV area at risk are shown in Figure 1. The areas at risk were similar for all groups, indicating that the anatomical regions perfused by the LCx were similar in all groups. Infarct size, expressed as the percent of the LV area at risk, was between 35% and 40% in vehicle-treated, nonpreconditioned dogs. Infarct size was significantly reduced by preconditioning to less than half of the nonpreconditioned sham value. Glyburide alone in nonpreconditioned animals did not significantly affect infarct size, although there was a slight tendency for an increase in infarct size. Despite this lack of effect of glyburide alone on infarct size, glyburide almost completely abolished the protective effect of preconditioning. A significant positive correlation existed between the LV area at risk and the LV infarct size in the control group (data not shown). The correlation was not as clear when the animals were preconditioned and the slope of the regression curve was significantly smaller in the preconditioned group. The regression equations for both of the groups treated with glyburide were similar to the control group.

The effect of preconditioning with or without glyburide on regional myocardial blood flow is shown in Table 1. No significant differences for baseline myocardial blood flows were noted between groups for any region measured. LCx occlusion caused a significant reduction in blood flow into the LCx perfused region, and the flow decrement was most severe in the subendocardial region. No differences in collateral blood flow were observed between any of the groups. During LCx occlusion, blood flow into the nonischemic region was slightly increased in all groups, but in general, this increase in flow was not significant. During reperfusion, flow into the previously ischemic region returned toward preischemic control levels in all groups. In Figure 2, the relation between infarct size as a percent of the area at risk and collateral flow into the inner half of the myocardium (subendocardium) for all groups is shown. A significant negative correlation was observed for the vehicle control group, and preconditioning significantly altered the regression line, indicating that at a given collateral flow, myocardial infarct size was reduced. The curves for both glyburide-treated groups were not significantly different from vehicle. The group given glyburide without preconditioning showed a slight increase

![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** Bar graphs show effect of intracoronary glyburide (GLY) on myocardial infarct size in preconditioned and nonpreconditioned dogs. Infarct size is expressed as a percent of the area at risk (similar for all groups). Infarct size was reduced by preconditioning (*p<0.05 vs. nonpreconditioned vehicle), and glyburide significantly (†p<0.05) increased infarct size relative to vehicle-treated, preconditioned dogs. LV, left ventricle.

a value of \( p<0.05 \) was accepted as significantly different. All values are expressed as mean±SEM.

**Results**

**Effect of Glyburide on Preconditioning**

The effect of glyburide and preconditioning on myocardial infarct size and LV area at risk are shown in Figure 1. The areas at risk were similar for all groups, indicating that the anatomical regions perfused by the LCx were similar in all groups. Infarct size, expressed as the percent of the LV area at risk, was between 35% and 40% in vehicle-treated, nonpreconditioned dogs. Infarct size was significantly reduced by preconditioning to less than half of the nonpreconditioned sham value. Glyburide alone in nonpreconditioned animals did not significantly affect infarct size, although there was a slight tendency for an increase in infarct size. Despite this lack of effect of glyburide alone on infarct size, glyburide almost completely abolished the protective effect of preconditioning. A significant positive correlation existed between the LV area at risk and the LV infarct size in the control group (data not shown). The correlation was not as clear when the animals were preconditioned and the slope of the regression curve was significantly smaller in the preconditioned group. The regression equations for both of the groups treated with glyburide were similar to the control group.

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**Table 1. Effect of Glyburide and/or Preconditioning on Regional Myocardial Blood Flow Before and After Myocardial Ischemia**

<table>
<thead>
<tr>
<th></th>
<th>Nonoccluded region</th>
<th>1 Hour after reperfusion</th>
<th>Occluded region</th>
<th>40 Minutes into LCx occlusion</th>
<th>1 Hour after reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 40 Minutes into LCx occlusion</td>
<td>135±18</td>
<td>35±18</td>
<td>121±15</td>
<td>21±8*</td>
</tr>
<tr>
<td>Subepicardium (ml/min/100 g)</td>
<td>Vehicle</td>
<td>149±18</td>
<td>146±34</td>
<td>135±28</td>
<td>3±1*</td>
</tr>
<tr>
<td></td>
<td>Vehicle preconditioning</td>
<td>181±13</td>
<td>125±14</td>
<td>110±5</td>
<td>5±2*</td>
</tr>
<tr>
<td></td>
<td>Glyburide</td>
<td>121±17</td>
<td>140±15</td>
<td>133±35</td>
<td>2±1*</td>
</tr>
<tr>
<td></td>
<td>Glyburide+preconditioning</td>
<td>110±8</td>
<td>148±13*</td>
<td>130±7</td>
<td>3±2*</td>
</tr>
<tr>
<td>Subendocardium (ml/min/100 g)</td>
<td>Vehicle</td>
<td>149±18</td>
<td>146±34</td>
<td>135±28</td>
<td>3±1*</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>Glyburide+preconditioning</td>
<td>110±8</td>
<td>148±13*</td>
<td>130±7</td>
<td>3±2*</td>
</tr>
</tbody>
</table>

All values are mean±SEM. LCx, left circumflex coronary artery.

*Significantly different from its respective control value (\( p<0.05 \)).
Figure 2. Regression curves for the relation between left ventricular infarct size expressed as a percent of the area at risk (LVAR) and subendocardial collateral flow into the ischemic region perfused by the left circumflex coronary artery. GLY, glyburide.

in the y-intercept, although this was not significantly different from vehicle ($\alpha=0.41$).

Hemodynamic data for all groups are shown in Table 2. None of the drug or vehicle treatments resulted in significant changes in heart rate. Ischemia and reperfusion also did not alter heart rate in any of the groups studied. Both systolic and diastolic arterial blood pressures were unchanged throughout the experiment in all groups. No significant differences in blood pressure were observed between any of the groups.

**Effect of Glyburide on the Cardioprotective Action of R-PIA**

Hemodynamic data for all groups in this series are shown in Table 3. Baseline heart rates and arterial blood pressures were similar for all groups before drug treatment or ischemia. No changes in heart rate were observed in any group after drug treatment. Ischemia did not significantly alter heart rate in any group. In all groups, heart rate tended to increase with time after reperfusion, although the increases did not achieve statistical significance. Treatment with R-PIA with or without glyburide did not affect arterial blood pressure before or during ischemia. Systolic and diastolic blood pressures were significantly reduced late into reperfusion, and similar reductions were observed for all treatment groups.

Myocardial infarct size and area at risk data for all groups are shown in Figure 3. LV area at risk was found to be similar in all groups, indicating similar anatomical regions. R-PIA was found to significantly reduce infarct size as a percent of the area at risk. Glyburide completely abolished this protective effect. The dose of glyburide used was found to have no effect, when given alone, on infarct size in the preceding series of experiments. A significant correlation between infarct size and area at risk was observed in vehicle control animals (data not shown). This was also true for R-PIA plus glyburide. The relation for R-PIA alone was not as clear, as a much smaller degree of variability in infarct size was accounted for by area at risk. The R-PIA curve was significantly shifted to the right relative to vehicle controls, and both the y-intercept and slope were significantly different. The relation between LV infarct size as a percent of the area at risk and subendocardial collateral flow is shown in Figure 4. A significant relation was observed for vehicle controls, and a similar

**Table 2. Effect of Glyburide and/or Preconditioning on Hemodynamic Variables Before and After Myocardial Ischemia**

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (beats per minute)</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Diastolic blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>153±9</td>
<td>116±6</td>
<td>94±6</td>
</tr>
<tr>
<td>20 Minutes after drug</td>
<td>152±9</td>
<td>120±8</td>
<td>97±7</td>
</tr>
<tr>
<td>LCx occlusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Minutes</td>
<td>158±7</td>
<td>113±10</td>
<td>90±8</td>
</tr>
<tr>
<td>60 Minutes</td>
<td>158±7</td>
<td>117±7</td>
<td>97±6</td>
</tr>
<tr>
<td>Hour into reperfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>149±9</td>
<td>109±8</td>
<td>87±7</td>
</tr>
<tr>
<td>3</td>
<td>164±7</td>
<td>119±6</td>
<td>84±6</td>
</tr>
<tr>
<td>5</td>
<td>163±8</td>
<td>114±6</td>
<td>80±6</td>
</tr>
</tbody>
</table>

All values are mean±SEM. LCx, left circumflex coronary artery.
TABLE 3. Effect of R-PIA With or Without Glyburide on Hemodynamic Status in Anesthetized Dogs

<table>
<thead>
<tr>
<th>Heart rate (beats per minute)</th>
<th>Control</th>
<th>20 Minutes after drug</th>
<th>LCx occlusion (60 minutes)</th>
<th>Hour into reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>159±17</td>
<td>156±11</td>
<td>155±14</td>
<td>150±15 152±10 174±16</td>
</tr>
<tr>
<td>R-PIA</td>
<td>150±7</td>
<td>153±4</td>
<td>150±4</td>
<td>142±5 155±5 171±5</td>
</tr>
<tr>
<td>R-PIA+glyburide</td>
<td>154±7</td>
<td>154±4</td>
<td>157±6</td>
<td>150±4 168±10 164±7</td>
</tr>
</tbody>
</table>

Systolic blood pressure (mm Hg)

| Vehicle                        | 142±8   | 135±7                 | 122±9                     | 113±3 110±5 110±7    |
| R-PIA                          | 132±7   | 127±7                 | 110±5                     | 102±4 105±4 102±3    |
| R-PIA+glyburide                | 125±8   | 124±9                 | 115±7                     | 105±3 109±7 105±4    |

Diastolic blood pressure (mm Hg)

| Vehicle                        | 123±7   | 112±6                 | 104±8                     | 95±3* 90±5* 85±5*    |
| R-PIA                          | 112±5   | 110±6                 | 94±4                      | 86±5 85±4* 80±3*     |
| R-PIA+glyburide                | 110±7   | 105±7                 | 91±2                      | 87±3 87±10 83±5*     |

All values are mean±SEM. LCx, left circumflex coronary artery.

*Significantly different from its respective preischemic control value (p<0.05).

Relation was found for R-PIA plus glyburide. Little correlation was found to exist between LV infarct size and subendocardial blood flow for R-PIA–treated animals. The curve for R-PIA was significantly different from vehicle controls such that at a given subendocardial collateral flow, LV infarct size was smaller.

Regional myocardial blood flows for these animals are shown in Table 4. No differences in preischemic myocardial blood flow were observed between any groups. Blood flow into the LCx perfused region was significantly reduced during ischemia, particularly in the subendocardial region, and no differences in this collateral flow were observed for any group. At 1 hour into reperfusion, blood flow into the LCx region of vehicle-treated hearts was only partially restored, the reflow into the subendocardium being the lowest. In R-PIA–treated hearts, reflow into the LCx region returned nearly to preischemic values, and this increase was significantly higher in the subendocardium and subepicardium relative to vehicle. Cotreatment with glyburide resulted in a loss of the improved reflow observed for R-PIA alone.

Discussion

Brief periods of myocardial ischemia have been shown to result in an increased resistance of the affected region to subsequent, more severe episodes of ischemia.1-3 This effect, termed preconditioning, has been shown in dog, rabbit, pig, and rat hearts.1-3,6 Preconditioning can be observed in hearts subjected to only several minutes of ischemia; however, the protective effects appear to last several hours.17 The protective mechanism of action does not appear to result from an increased collateral flow or from reduced oxygen demand secondary to contractile stunning.1 Recent studies have indicated that activation of adenosine A1-receptors may be responsible for the preconditioning effect.3,5 This is based on the reversal of preconditioning by selective A1-receptor blockers3 and the protective effects of A1-agonists.3 Presently, the exact mechanism in which A1-activation can result in protection is not well understood, although preconditioning is associated with a reduced ATP utilization or a metabolite accumulation.17

Recent studies by Kirsch et al17 have indicated that KATP can be opened by A1-activation via a Gi protein–coupled mechanism. These interesting results indicate the possibility that A1-stimulation during preconditioning may open KATP and thus protect the ischemic hearts. Studies from several different laboratories have indicated that pharmacological activation of myocardial KATP is associated with profound cardioprotective effects in a variety of in vivo and in vitro models of
myocardial ischemia. The cardioprotective effects of these compounds (e.g., cromakalim, pinacidil) appear to be secondary to direct effects on the myocardium, whereas the vasodilatory effects seem to be unnecessary. KATP opener-induced cardioprotection is associated with preservation of ATP, which is similar to the observation of ATP preservation for preconditioning. Another interesting aspect of KATP activators is that their potency appears to be enhanced under ischemic conditions. It is possible that adenosine release during ischemia may alter the gating characteristics of KATP such that the apparent potency of the KATP openers is enhanced. Because adenosine is released during ischemia, it is possible that adenosine may alter the gating of KATP such that they are more readily opened by activators such as cromakalim.

To establish a link between preconditioning and KATP activation, Gross's group has shown that the KATP blockers glyburide and sodium 5-hydroxydecanoate completely abolish preconditioning in a canine model of ischemia and reperfusion. The doses of the blockers used in these studies were found to have no effect on infarct size when given to animals not subjected to preconditioning. Conversely, Thornton et al found that glyburide did not abolish preconditioning in rabbits despite a proischemic effect observed when it was given to non preconditioned animals. They also could not show a protective effect for the KATP opener pinacidil, unlike studies from our laboratory and others. At the present time, it is difficult to reconcile the results in rabbits with the results observed in dogs. It was of interest that in the rabbit study, glyburide alone was found to result in a significant proischemic effect but still did not abolish preconditioning. If glyburide was proischemic, the interesting question was why the KATP opener pinacidil did not protect the ischemic myocardium, unless the dose used was too low. Although it is possible to speculate that glyburide may have other activities that may make it proischemic, studies have indicated that glyburide is selective in blocking KATP in ischemic myocardium. It is also possible that species differences may exist.

We thus considered the question of the role of KATP in preconditioning worth reexamining. We chose the dog because of its known response to preconditioning and also because more is known about its myocardial KATP compared with species such as the rabbit. We determined the ability of glyburide to abolish preconditioning in the present study. We found that a 5-minute period of LCx occlusion resulted in a significant protective effect when the same region was exposed to a subsequent 60-minute period of occlusion. As shown previously by several investigators, this protective effect was not accompanied by an increase in collateral flow into either the subendocardium or subepicardium. Glyburide was given in a dose that when given to non preconditioned dogs had little effect on the outcome of the ischemic event relative to vehicle-

![Graph showing correlation between left ventricular (LV) infarct size as a percent of the area at risk and subendocardial collateral flow in animals treated with vehicle (CONTROL), R-PIA (A1-agonist), or R-PIA plus glyburide.](image)

**FIGURE 4.** Graphs show correlation between left ventricular (LV) infarct size as a percent of the area at risk and subendocardial collateral flow in animals treated with vehicle (CONTROL), R-PIA (A1-agonist), or R-PIA plus glyburide.

**TABLE 4.** Effect of R-PIA With or Without Glyburide on Regional Myocardial Blood Flow in Anesthetized Dogs

<table>
<thead>
<tr>
<th></th>
<th>Nonoccluded region</th>
<th>Occluded region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>40-Minute</td>
</tr>
<tr>
<td><strong>Subepicardium (ml/min/100 g)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Vehicle</td>
<td>110±11</td>
<td>123±15</td>
</tr>
<tr>
<td>R-PIA</td>
<td>108±15</td>
<td>111±8</td>
</tr>
<tr>
<td>R-PIA + glyburide</td>
<td>96±14</td>
<td>93±12</td>
</tr>
<tr>
<td><strong>Subendocardium (ml/min/100 g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>125±15</td>
<td>126±27</td>
</tr>
<tr>
<td>R-PIA</td>
<td>111±17</td>
<td>107±12</td>
</tr>
<tr>
<td>R-PIA + glyburide</td>
<td>103±19</td>
<td>103±12</td>
</tr>
</tbody>
</table>

All values are mean±SEM.

*Significantly different from its respective preischemic control value (p<0.05).
†Significantly different from its respective vehicle-treated group value (p<0.05).
treated animals. Certainly, any slight prosiemic effect of glyburide could not account for its almost complete reversal of preconditioning. A prosiemic effect of glyburide in nonpreconditioned dogs cannot be excluded in our study due to the small number of animals studied, i.e., we cannot exclude a type II statistical error, \( \alpha = 0.41 \). In preconditioned animals, glyburide abolished the protective effect observed after brief LCx occlusion. This effect of glyburide was not accompanied by a significant alteration in collateral flow. When infarct sizes were compared as a function of collateral blood flow, preconditioning still exerted a protective effect, and glyburide abolished this effect. Thus, our data are in agreement with the study from Gross’s laboratory with glyburide\(^4\) and the collaborative study between Gross’s laboratory and our laboratory using the \( K_{\text{ATP}} \) blocker sodium 5-hydroxydecanoate.\(^12\)

We are confident that in the dog, \( K_{\text{ATP}} \) activation can mediate at least some of the protective effect of preconditioning. We also attempted to link the work indicating \( A_1 \)-stimulation and work indicating \( K_{\text{ATP}} \) activation in mediating preconditioning. Studies from several laboratories have shown that \( A_1 \)-antagonists can abolish preconditioning in several species, including the dog.\(^5,22\) Gross and Auchampach\(^22\) showed that an \( A_1 \)-receptor antagonist abolishes preconditioning in a canine model of preconditioning that is virtually identical with our model. We extended this observation by determining the effect of glyburide on the cardioprotective effects of the \( A_1 \)-agonist R-PIA. We did not determine the effect of \( A_1 \)-antagonists on preconditioning, as this has been done by several investigators (unlike the effect of glyburide, which was more controversial). Like several previous studies,\(^3,23,24\) we have shown R-PIA to reduce infarct size, and this appears to be protective despite a lack of effects on collateral flow. Several studies have indicated that adenosine may have protective effects in several models of ischemia,\(^14,23\) as have agents that enhance the adenosine action such as adenosine deaminase inhibitors.\(^25\) Proposed protective mechanisms include inhibitory effects on neutrophil function,\(^26\) enhancement of reflow,\(^27\) or a metabolic effect.\(^23\) To our knowledge, no investigator has yet determined a direct link between \( A_1 \)-receptor–induced cardioprotection and \( K_{\text{ATP}} \) activation. The results from Downey’s laboratory\(^5\) indicating that preconditioning can be abolished by pertussis toxin and the results from Kirsch et al\(^7\) indicating that \( A_1 \)-receptors and \( K_{\text{ATP}} \) are linked by a \( G_i \) protein show that our study on the effect of glyburide on \( A_1 \)-cardioprotection is a logical step.

**Summary**

Activation of \( K_{\text{ATP}} \) may mediate the protective effect of myocardial preconditioning, \( K_{\text{ATP}} \) may be activated by the release of adenosine (or another endogenous agonist of \( A_1 \)-receptors) induced by preconditioning. These results suggest the therapeutic potential of \( K_{\text{ATP}} \) openers or agents that can stimulate \( A_1 \)-receptors for the treatment of acute myocardial ischemia.

**References**


5. Schwarz ER, Mohri M, Sack S, Arras M: The role of adenosine and its \( A_1 \)-receptor in ischemic preconditioning. (abstract) Circulation 1991;84(suppl II):II-191

6. Thornton J, Downey JM: \( G \), proteins are involved in preconditioning’s protective effects. (abstract) Circulation 1991;84(suppl II):II-192


24. Tsuchida A, Miura T, Imura O: Role of adenosine receptor activation in infarct size limitation by preconditioning in the heart. (abstract) Circulation 1991;84(suppl II):II-191


Role of myocardial ATP-sensitive potassium channels in mediating preconditioning in the
dog heart and their possible interaction with adenosine A1-receptors.
G J Grover, P G Sleph and S Dzwonczyk

Circulation. 1992;86:1310-1316
doi: 10.1161/01.CIR.86.4.1310

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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the World Wide Web at:
http://circ.ahajournals.org/content/86/4/1310

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